U.S. Application No.
Unknown

International Application No.
PCT/AU99/00691

Attorney Docket No.
DAVI121.001APC

Date: February 27, 2001

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 USC 371

International Application No.:	PCT/AU99/00691
International Filing Date:	26 August 1999
Priority Date Claimed:	27 August 1998
Title of Invention:	NOVEL THERAPEUTIC MOLECULES AND USES THEREFOR
Applicant(s) for DO/EO/US:	Ismail Kola and Jiong Zhou

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. (X) This is a **FIRST** submission of items concerning a filing under 35 USC 371.

2. (X) This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Articles 22 and 39(1).

3. (X) A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

4. (X) A copy of the International Application as filed (35 USC 371(c)(2))

   a) () is transmitted herewith (required only if not transmitted by the International Bureau).
   b) (X) has been transmitted by the International Bureau.
   c) () is not required, as the application was filed in the United States Receiving Office (RO/US).

5. (X) Amendments to the claims of the International Application under PCT Article 19 (35 USC 371(c)(3))

   a) (X) are transmitted herewith (required only if not transmitted by the International Bureau).
   b) () have been transmitted by the International Bureau.
   c) () have not been made; however, the time limit for making such amendments has NOT expired.
   d) () have not been made and will not be made.

6. (X) A copy of the International Preliminary Examination Report with any annexes thereto, such as any amendments made under PCT Article 34.

Items 11. to 16. below concern other document(s) or information included:

7. (X) International Application as published (Cover sheet only).

8. (X) PCT Form PCT/IPEA/402.

9. (X) PCT Form PCT/IB/308.

10. (X) A return prepaid postcard.

11. (X) The following fees are submitted:

				FEES
	BASIC FEE			$1,000
CLAIMS	**NUMBER FILED**	**NUMBER EXTRA**	**RATE**	
Total Claims	76 - 20 =	56 ×	$18	$1008
Independent Claims	25 - 3 =	22 ×	$80	$1760
Multiple dependent claims(s) (if applicable)			$270	$0
	TOTAL OF ABOVE CALCULATIONS		$3768	
Reduction by 1/2 for filing by small entity (if applicable). Verified Small Entity statement must also be filed. (NOTE 37 CFR 1.9, 1.27, 1.28)			$	
	TOTAL NATIONAL FEE			$3768
	TOTAL FEES ENCLOSED			$3768
		amount to be refunded:		$
		amount to be charged:		$

12. (X) The fee for later submission of the signed oath or declaration set forth in 37 CFR 1.492(e) will be paid upon submission of the declaration.

13. (X) A check in the amount of $3768 to cover the above fees is enclosed.

14. (X) The Commissioner is hereby authorized to charge only those additional fees which may be required, now or in the future, to avoid abandonment of the application, or credit any overpayment to Deposit Account No. 11-1410. A duplicate copy of this sheet is enclosed.

15. (X) Preliminary Amendment.

16. (X) Copy of International Search Report.

17. (X) Copy of Demand.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

KNOBBE, MARTENS, OLSON & BEAR, LLP
620 Newport Center Drive
Sixteenth Floor
Newport Beach, CA 92660

Signature

Sam K. Tahmassebi
Printed Name

45,151
Registration Number

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 PCT

PATENT

Case Docket No. DAVI121.001APC
Date: April 11, 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Ismail Kola
Appl. No.	:	09/786,043
Filed	:	February 27, 2001
For	:	NOVEL THERAPEUTIC MOLECULES AND USES THEREFOR
Examiner	:	Not yet assigned
Group Art Unit	:	Not yet assigned

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on

April 11, 2001
(Date)

Sam K. Tahmassebi, Reg. No. 45,151

TRANSMITTAL LETTER

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231
ATTENTION: APPLICATION BRANCH

Dear Sir:

Enclosed for filing in the above-identified application are:

(X) Second Preliminary Amendment.

(X) The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Account No. 11-1410.

(X) Return prepaid postcard.

Sam K. Tahmassebi
Registration No. 45,151
Attorney of Record

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DAVI121.001APC PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Ismail Kola	Group Art Unit Not yet assigned
Appl. No.	:	Not yet assigned	
Filed	:	Herewith	
For	:	**NOVEL THERAPEUTIC MOLECULES AND USES THEREFOR**	
Examiner	:	Not yet assigned	

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C 20231, on

February 27, 2001
(Date)

Sam K. Tahmassebi, Reg No 45,151

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to the examination on the merits of the captioned application, please enter the following amendments.

IN THE SPECIFICATION:

At page 1, line 2, please insert:

-- RELATED APPLICATIONS

This application is national phase of International Application No. PCT/AU99/00691, filed on August 26, 1999; which claimed priority from Australian Application No. PP 5512, filed on August 27, 1998.--

IN THE CLAIMS:

Please cancel all the pending claims 1-50.

Please add the following new claims 51-108:

51. (NEW) An isolated nucleic acid molecule comprising a nucleotide sequence encoding ELF5 wherein said ELF5 comprises an Ets domain.

52. (NEW) An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>2 or a derivative homologue or mimetic thereof or having at least about 45% or greater similarity to at least 10 contiguous amino acids in <400>2.

53. (NEW) An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence substantially as set forth in <400>1 or a derivative or homologue thereof capable of hybridizing to <400>1 under low stringency conditions.

54. (NEW) An isolated nucleic acid molecule according to claim 61 which further encodes an amino acid sequence substantially as set forth in <400>2 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>2.

55. (NEW) An isolated nucleic acid molecule according to claim 60 substantially as set forth in <400>1.

56. (NEW) An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>4 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to at least 10 contiguous amino acids in <400>4.

57. (NEW) An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence substantially as set forth in <400>3 or a derivative or homologue thereof capable of hybridizing to <400>3 under low stringency conditions.

58. (NEW) An isolated nucleic acid molecule according to claim 65 which further encodes an amino acid sequence substantially as set forth in <400>4 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>4.

59. (NEW) An isolated nucleic acid molecule according to claim 64 substantially as set forth in <400>3.

60. (NEW) An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>7 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to at least 10 contiguous amino acids in <400>7.

61. (NEW) An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence substantially as set forth in one of <400>5 or <400>6 or a derivative or homologue thereof capable of hybridizing to one of <400>5 or <400>6 under low stringency conditions.

62. (NEW) An isolated nucleic acid molecule according to claim 69 which further encodes an amino acid sequence corresponding to an amino acid sequence set forth in <400>7 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>7.

63. (NEW) An isolated nucleic acid molecule according to claim 68 substantially as set forth in <400>5 or <400>6.

64. (NEW) An isolated protein or derivative, homologue, analogue, chemical equivalent or mimetic thereof wherein said protein is ELF5 which ELF5 comprises an Ets domain.

65. (NEW) An isolated protein comprising an amino acid sequence substantially as set forth in <400>2 or a derivative, homologue or mimetic thereof or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>2 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

66. (NEW) An isolated protein according to claim 73 encoded by a nucleotide sequence substantially as set forth in <400>1 or a derivative, homologue or analogue thereof or capable of hybridizing to <400>1 under low stringency conditions or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

67. (NEW) An isolated protein according to claim 73 substantially as set forth in <400>2.

68. (NEW) An isolated protein having an amino acid sequence substantially as set forth in <400>4 or a derivative, homologue or mimetic thereof or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>4 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

69. (NEW) An isolated protein according to claim 76 encoded by a nucleotide sequence substantially as set forth in <400>3 or a derivative, homologue or mimetic thereof or capable of hybridizing to <400>3 under low stringency conditions or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

70. (NEW) An isolated protein according to claim 76 substantially as set forth in <400>4.

71. (NEW) An isolated protein comprising an amino acid sequence substantially as set forth in <400>7 or a derivative, homologue or mimetic thereof or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>7 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

72. (NEW) An isolated protein according to claim 79 encoded by a nucleotide sequence substantially as set forth in one of <400>5 or <400>6 or a derivative, homologue or mimetic thereof or capable of hybridizing to one of <400>5 or <400>6 under low stringency conditions or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

73. (NEW) An isolated protein according to claim 79 substantially as set forth in <400>7.

74. (NEW) An isolated protein according to claim 72 which protein is a homodimer.

75. (NEW) An isolated protein according to claim 72 which protein is a heterodimer.

76. (NEW) A method of modulating expression of *ELF5* in a mammal, said method comprising contacting the *ELF5* gene with an effective amount of an agent for a time and under conditions sufficient to modulate expression of *ELF5*.

77. (NEW) A method of modulating the functional activity of ELF5 in a mammal, said method comprising administering to said mammal a modulating effective amount of an agent for a time and under conditions sufficient to increase or decrease the ELF5 activity.

78. (NEW) A method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of *ELF5* or sufficient to modulate the activity of ELF5.

79. (NEW) A method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of a protein according to claim 72 or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate the functional activity of said cell.

80. (NEW) A method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of a nucleic acid molecule according to claim 59 or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate the functional activity of said cell.

81. (NEW) A method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of *ELF5* or sufficient to modulate the activity of ELF5 wherein said *ELF5* expression product or ELF5 modulates the activity of said cell.

82. (NEW) A method according to any one of claim 86 wherein said functional activity is proliferation.

83. (NEW) A method according to claim 90 wherein said cell is a neoplastic epithelial cell and said modulation is down-regulation.

84. (NEW) A method according to claim 91 wherein said neoplastic epithelial cell is of breast, prostate or lung origin.

85. (NEW) A method for the treatment and/or prophylaxis of a condition characterized by the aberrant, unwanted or otherwise inappropriate cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of an

agent for a time and under conditions sufficient to modulate the expression of *ELF5* wherein said modulation results in modulation of cellular functional activity.

86. (NEW) A method for the treatment and/or prophylaxis of a condition characterized by the aberrant, unwanted or otherwise inappropriate cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the activity of ELF5 wherein said modulation results in modulation of cellular functional activity.

87. (NEW) A method for the treatment and/or prophylaxis of a condition characterized by the aberrant, unwanted or otherwise inappropriate cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of a protein according to claim 72 or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate cellular functional activity.

88. (NEW) A method for the treatment and/or prophylaxis of a condition characterized by the aberrant, unwanted or otherwise inappropriate cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of a nucleic acid molecule according to claim 59 or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate cellular functional activity.

89. (NEW) A method of treating a mammal according to any one of claims 93-96 wherein said condition is an epithelial cell malignancy.

90. (NEW) A method according to claim 97 wherein said malignant epithelial cell is of breast, prostate or lung origin.

91. (NEW) A method according to claim 97 wherein said functional activity is proliferation and said modulation is down-regulation.

92. (NEW) A pharmaceutical composition comprising *ELF5*, ELF5 or an agent capable of modulating *ELF5* expression or ELF5 activity or derivative, homologue, analogue, chemical equivalent or mimetic thereof together with one or more pharmaceutically acceptable carriers and/or diluents.

93. (NEW) An isolated antibody directed to the protein according to claim 72.

94. (NEW) An isolated antibody directed to the nucleic acid molecule according to claim 59.

95. (NEW) The antibody according to claim 101 wherein said antibody is a monoclonal antibody.

96. (NEW) The antibody according to claim 101 wherein said antibody is a polyclonal antibody.

97. (NEW) A method of diagnosing or monitoring a mammalian disease condition, which disease condition is characterized by aberrant cellular functional activity, said method comprising screening for ELF5 or *ELF5* in a biological sample isolated from said mammal.

98. (NEW) A method for detecting an agent capable of modulating the function of ELF5 or its functional equivalent or derivative thereof said method comprising contacting a cell or extract thereof containing said ELF5 or its functional equivalent or derivative with a putative agent and detecting an altered expression phenotype associated with said ELF5 or its functional equivalent or derivative.

99. (NEW) A method for detecting an agent capable of modulating the function of ELF5 or its functional equivalent or derivative thereof said method comprising contacting an epithelial cell containing said ELF5 or its functional equivalent or derivative with a putative agent and detecting an altered proliferation rate.

100. (NEW) A method for detecting an agent capable of binding or otherwise associating with an ELF5 binding site or functional equivalent or derivative thereof said method comprising contacting a cell containing said ELF5 binding site or functional equivalent or derivative thereof with a putative agent and detecting an altered expression phenotype associated with modulation of the function of ELF5 or its functional equivalent or derivative.

REMARKS

By present amendments, Applicants have incorporated into the specification a paragraph indicating that the present application is the national phase of the aforementioned International Application, to which the present application claims priority. Applicants have also included a new set of claims to be examined with this application. Applicants maintain that the new claims add no new matter and are fully supported by the specification and the priority document. By

these amendments, Applicants make no admission as to the patentability of the original claims and the amendments should not be so construed.

Applicants maintain that the claims as presented herewith are allowable. A notice to that effect is respectfully requested.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: Feb. 27, 2001

By: _____

Sam K. Tahmassebi
Registration No. 45,151
Attorney of Record
620 Newport Center Drive
Sixteenth Floor
Newport Beach, CA 92660

AMEND
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DAVI121.001APC

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Ismail Kola	Group Art Unit Not yet assigned
Appl. No.	:	09/786,043	
Filed	:	February 27, 2001	
For	:	**NOVEL THERAPEUTIC MOLECULES AND USES THEREFOR**	
Examiner	:	Not yet assigned	

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on

April 11, 2001
(Date)

Sam K. Tahmassebi, Reg. No. 45,151

SECOND PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to the examination on the merits of the captioned application, please enter the following amendments.

IN THE CLAIMS:

Please cancel all the pending claims 51-100.

Please add the following new claims 101-150:

101. (NEW) An isolated nucleic acid molecule comprising a nucleotide sequence encoding ELF5 wherein said ELF5 comprises an Ets domain.

102. (NEW) An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence encoding, or a nucleotide sequence

complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>2 or a derivative homologue or mimetic thereof or having at least about 45% or greater similarity to at least 10 contiguous amino acids in <400>2.

103. (NEW) An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence substantially as set forth in <400>1 or a derivative or homologue thereof capable of hybridizing to <400>1 under low stringency conditions.

104. (NEW) An isolated nucleic acid molecule according to Claim 103 which further encodes an amino acid sequence substantially as set forth in <400>2 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>2.

105. (NEW) An isolated nucleic acid molecule according to Claim 102 substantially as set forth in <400>1.

106. (NEW) An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>4 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to at least 10 contiguous amino acids in <400>4.

107. (NEW) An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence substantially as set forth in <400>3 or a derivative or homologue thereof capable of hybridizing to <400>3 under low stringency conditions.

108. (NEW) An isolated nucleic acid molecule according to Claim 107 which further encodes an amino acid sequence substantially as set forth in <400>4 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>4.

109. (NEW) An isolated nucleic acid molecule according to Claim 106 substantially as set forth in <400>3.

110. (NEW) An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>7 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to at least 10 contiguous amino acids in <400>7.

111. (NEW) An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence substantially as set forth in one of <400>5 or <400>6 or a derivative or homologue thereof capable of hybridizing to one of <400>5 or <400>6 under low stringency conditions.

112. (NEW) An isolated nucleic acid molecule according to Claim 111 which further encodes an amino acid sequence corresponding to an amino acid sequence set forth in <400>7 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>7.

113. (NEW) An isolated nucleic acid molecule according to Claim 110 substantially as set forth in <400>5 or <400>6.

114. (NEW) An isolated protein or derivative, homologue, analogue, chemical equivalent or mimetic thereof wherein said protein is ELF5 which ELF5 comprises an Ets domain.

115. (NEW) An isolated protein comprising an amino acid sequence substantially as set forth in <400>2 or a derivative, homologue or mimetic thereof or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>2 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

116. (NEW) An isolated protein according to Claim 115 encoded by a nucleotide sequence substantially as set forth in <400>1 or a derivative, homologue or analogue thereof or capable of hybridizing to <400>1 under low stringency conditions or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

117. (NEW) An isolated protein according to Claim 115 substantially as set forth in <400>2.

118. (NEW) An isolated protein having an amino acid sequence substantially as set forth in <400>4 or a derivative, homologue or mimetic thereof or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>4 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

119. (NEW) An isolated protein according to Claim 118 encoded by a nucleotide sequence substantially as set forth in <400>3 or a derivative, homologue or mimetic thereof or capable of hybridizing to <400>3 under low stringency conditions or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

120. (NEW) An isolated protein according to Claim 118 substantially as set forth in <400>4.

121. (NEW) An isolated protein comprising an amino acid sequence substantially as set forth in <400>7 or a derivative, homologue or mimetic thereof or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>7 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

122. (NEW) An isolated protein according to Claim 121 encoded by a nucleotide sequence substantially as set forth in one of <400>5 or <400>6 or a derivative, homologue or mimetic thereof or capable of hybridizing to one of <400>5 or <400>6 under low stringency conditions or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

123. (NEW) An isolated protein according to Claim 121 substantially as set forth in <400>7.

124. (NEW) An isolated protein according to Claim 114 which protein is a homodimer.

125. (NEW) An isolated protein according to Claim 114 which protein is a heterodimer.

126. (NEW) A method of modulating expression of *ELF5* in a mammal, said method comprising contacting the *ELF5* gene with an effective amount of an agent for a time and under conditions sufficient to modulate expression of *ELF5*.

127. (NEW) A method of modulating the functional activity of ELF5 in a mammal, said method comprising administering to said mammal a modulating effective amount of an agent for a time and under conditions sufficient to increase or decrease the ELF5 activity.

128. (NEW) A method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of *ELF5* or sufficient to modulate the activity of ELF5.

129. (NEW) A method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of a protein according to Claim 114 or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate the functional activity of said cell.

130. (NEW) A method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of a nucleic acid molecule according to Claim 101 or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate the functional activity of said cell.

131. (NEW) A method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of *ELF5* or sufficient to modulate the activity of ELF5 wherein said *ELF5* expression product or ELF5 modulates the activity of said cell.

132. (NEW) A method according to any one of Claim 128 wherein said functional activity is proliferation.

133. (NEW) A method according to Claim 132 wherein said cell is a neoplastic epithelial cell and said modulation is down-regulation.

134. (NEW) A method according to Claim 133 wherein said neoplastic epithelial cell is of breast, prostate or lung origin.

135. (NEW) A method for the treatment and/or prophylaxis of a condition characterized by the aberrant, unwanted or otherwise inappropriate cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of *ELF5* wherein said modulation results in modulation of cellular functional activity.

136. (NEW) A method for the treatment and/or prophylaxis of a condition characterized by the aberrant, unwanted or otherwise inappropriate cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the activity of ELF5 wherein said modulation results in modulation of cellular functional activity.

137. (NEW) A method for the treatment and/or prophylaxis of a condition characterized by the aberrant, unwanted or otherwise inappropriate cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of a protein according to Claim 114 or a derivative, homologue, analogue, chemical equivalent or

mimetic thereof for a time and under conditions sufficient to modulate cellular functional activity.

138. (NEW) A method for the treatment and/or prophylaxis of a condition characterized by the aberrant, unwanted or otherwise inappropriate cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of a nucleic acid molecule according to Claim 101 or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate cellular functional activity.

139. (NEW) A method of treating a mammal according to any one of Claims 135-138 wherein said condition is an epithelial cell malignancy.

140. (NEW) A method according to Claim 139 wherein said malignant epithelial cell is of breast, prostate or lung origin.

141. (NEW) A method according to Claim 139 wherein said functional activity is proliferation and said modulation is down-regulation.

142. (NEW) A pharmaceutical composition comprising *ELF5*, ELF5 or an agent capable of modulating *ELF5* expression or ELF5 activity or derivative, homologue, analogue, chemical equivalent or mimetic thereof together with one or more pharmaceutically acceptable carriers and/or diluents.

143. (NEW) An isolated antibody directed to the protein according to Claim 114.

144. (NEW) An isolated antibody directed to the nucleic acid molecule according to Claim 101.

145. (NEW) The antibody according to Claim 143 or 144 wherein said antibody is a monoclonal antibody.

146. (NEW) The antibody according to Claim 143 or 144 wherein said antibody is a polyclonal antibody.

147. (NEW) A method of diagnosing or monitoring a mammalian disease condition, which disease condition is characterized by aberrant cellular functional activity, said method comprising screening for ELF5 or *ELF5* in a biological sample isolated from said mammal.

~~148.~~ (NEW) A method for detecting an agent capable of modulating the function of ELF5 or its functional equivalent or derivative thereof said method comprising contacting a cell or extract thereof containing said ELF5 or its functional equivalent or derivative with a putative agent and detecting an altered expression phenotype associated with said ELF5 or its functional equivalent or derivative.

149. (NEW) A method for detecting an agent capable of modulating the function of ELF5 or its functional equivalent or derivative thereof said method comprising contacting an epithelial cell containing said ELF5 or its functional equivalent or derivative with a putative agent and detecting an altered proliferation rate.

150. (NEW) A method for detecting an agent capable of binding or otherwise associating with an ELF5 binding site or functional equivalent or derivative thereof said method comprising contacting a cell containing said ELF5 binding site or functional equivalent or derivative thereof with a putative agent and detecting an altered expression phenotype associated with modulation of the function of ELF5 or its functional equivalent or derivative.

REMARKS

The above Claims 101-150 represent all the pending claims in the present application. By these amendments, Applicants correct a mistake generated by the previous Preliminary Amendment, through which two sets of Claims 50-58 remained pending before the Office, and no Claims 101-108 appeared in the amendments. By these amendments, Applicants make no admission as to the patentability of the original claims and the amendments should not be so construed.

Applicants maintain that the claims as presented herewith are allowable. A notice to that effect is respectfully requested.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: April 11, 2001

By: _____

Sam K. Tahmassebi
Registration No. 45,151
Attorney of Record
620 Newport Center Drive
Sixteenth Floor
Newport Beach, CA 92660

AMEND
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041001

NOVEL THERAPEUTIC MOLECULES AND USES THEREFOR

FIELD OF THE INVENTION

The present invention relates generally to novel molecules capable of, *inter alia*, controlling cellular functional activity such as proliferation, differentiation and/or transcriptional regulation and to genetic sequences encoding same. More particularly, the present invention relates to novel members of the ETS family of proteins, referred to herein as "ELF5", and to genetic sequences encoding same. The molecules of the present invention are useful, for example, in therapy, diagnosis, antibody generation and as a screening tool for agents capable of modulating transcriptional events during cellular functioning such as in tumorigenesis.

BACKGROUND OF THE INVENTION

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description.

The ETS family of transcription factors share a conserved DNA binding domain, termed the 'ETS domain', first identified in the *gag-myb-ets* fusion protein of avian leukemia virus E26 (Nunn *et al.*, 1983; Watson *et al.*, 1988; Karim *et al.*, 1990; Gutman and Wasylyk, 1991; Seth *et al.*, 1992). The ETS domain recognises and binds to purine rich GGA(A/T) core motifs in the promoters and enhancers of various target genes (Macleod *et al.*, 1992; Wasylyk *et al.*, 1993; Janknecht and Nordheim, 1993; Werner *et al.*, 1995; Kodandapani *et al.*, 1996). The ETS family does not maintain overall similarity outside of the ETS domain, but can be grouped into subfamilies based upon variation within the ETS domain, and also by the arrangement and presence of other domains, such as those involved in transactivation and sites of phosphorylation (Lautenberger *et al*, 1992; Wasylyk *et al.*, 1993; Janknecht and Nordheim, 1993). Over 30 ETS gene family members have been identified in species ranging from sea urchin to human.

Many ETS factors have been implicated in the control of cellular proliferation and tumorigenesis (Seth *et al.*, 1992; Macleod *et al.*, 1992; Wasylyk *et al.*, 1993; Janknecht and Nordheim, 1993; Scott *et al.*, 1994a; Muthusamy *et al.*, 1995). *ETS1, ETS2, ERG2* and *PU.1* are proto-oncogenes with mitogenic and transforming activity when overexpressed in fibroblasts (Seth *et al.*, 1989; Seth and Papas, 1990; Hart *et al.*, 1995; Moreau-Gachelin *et al.*, 1996). In addition, chromosomal translocations involving ETS family members are associated with different human cancers. *ERG* and *ERGB/FLI1* are fused to the *EWS* gene in t(21;22) and t(11;22) translocations, respectively, in Ewing's sarcoma and other primitive neuroectodermal tumors (Sorensen *et al.*, 1994; Ida *et al.*, 1995). *FEV* is fused to *EWS* in a subset of Ewing's tumors in t(2;22) (Peter *et al.*, 1997). *TEL* is fused to the platelet-derived growth factor receptor beta (PDGFRβ) gene in t(5;12) translocations of chronic myelomonocytic leukemia, and to the acute myeloid leukemia 1 (AML1) transcription factor gene in t(12;21) translocations of acute lymphoblastic leukemia (Golub *et al.*, 1994, 1995). Fusion of *TEL* to the receptor-associated kinase JAK2 results in early pre-B acute lymphoid leukemia in t(9;12), and in a typical chronic myelogenous leukemia in t(9;15;12) (Peeters *et al.*, 1997). Expression of *Spi1* and *Fli1* can be activated by position specific integration of the Friend murine leukemia virus in murine erythroleukemias (Ben-David *et al.*, 1991). Also, ETS1, ETS2 and ERG regulate the expression of metalloproteinase genes, such as stromelysin and collagenase (Buttice and Kurkinen, 1993; Buttice *et al.*, 1996; Wasylyk *et al.*, 1991), which are important for extracellular matrix degradation concomitant with tumor vascularization (angiogenesis) and metastasis.

ETS factors also have important developmental roles. *Pointed P2* and *yan* play critical roles in *Drosophila* eye development (O'Neill *et al.*, 1994). *ETS2* is involved in skeletal/cartilage development (Sumarsono *et al.*, 1996). *PU.1* null mutation results in haematopoietic abnormalities (McKercher *et al.*, 1996), and *ETS1* is involved in transactivation of genes required for T cell function (Muthusamy *et al.*, 1995; Sun *et al.*, 1995; Thomas *et al.*, 1995; Thomas *et al.*, 1997) and angiogenesis (Wasylyk *et al.*, 1991; Vandenbunder *et al.*, 1994; Wernert *et al.*, 1992).

The ETS factors are almost all expressed in haematopoietic lineages (Bhat *et al.*, 1989; Bhat *et al.*, 1990; Kola *et al.*, 1993), and indeed appear to function predominantly in these cells and their related neoplasms. However, the most common solid tumors in humans are carcinomas which arise from the transformation of epithelial cells. Transformed breast epithelial cells, for example, have been shown to express ETS family members GABPα, PEA3, ELF1, ETS1 and ELK1 (Scott *et al.*, 1994b; Delannoy-Courdent *et al.*, 1996), but expression of these ETS family members is not restricted to epithelial cells. One ETS family member, ELF3/ESX/ESE-1/ERT, has recently emerged with epithelial and epithelial-cancer specific expression (Tymms *et al.*, 1997; Chang *et al.*, 1997; Choi *et al.*, 1998; Oettgen *et al.*, 1997).

In work leading up to the present invention, the inventors have identified and sequenced a novel member of the ETS family, designated herein "ELF5".

SUMMARY OF THE INVENTION

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The subject specification contains nucleotide and amino acid sequence information prepared using the programme PatentIn Version 2.0, presented herein after the bibliography. Each nucleotide or amino acid sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (e.g. <210>1, <210>2, etc). The length, type of sequence (DNA, protein (PRT), etc) and source organism for each nucleotide or amino acid sequence are indicated by information provided in the numeric indicator fields <211>, <212> and <213>, respectively. Nucleotide and amino sequences referred to in the specification are defined by the information provided in numeric indictor field <400> followed by the sequence identifier (e.g. <400>1, <400>2, etc).

One aspect of the present invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding ELF5 wherein said ELF5 comprises an ETS domain.

Another aspect of the present invention provides a nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>2 or <400>4 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to at least 10 contiguous amino acids in <400>2 or <400>4.

Yet another aspect of the present invention provides a nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>7 or a derivative, homologue or mimetic or having at least about 45% or greater similarity to at least 10 contiguous amino acids in <400>7.

Still yet another aspect of the present invention contemplates a nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence substantially as set forth in <400>1 or <400>3 or a derivative or homologue thereof capable of hybridising to one of <400>1 or <400>3 under low stringency conditions and which encodes an amino acid sequence corresponding to an amino acid sequence set forth in one of <400>2 or <400>4 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in one of <400>2 or <400>4.

A further aspect of the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in <400>1 or <400>3.

Another further aspect of the present invention contemplates a nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence substantially as set forth in one of <400>5 or <400>6 or a derivative or homologue thereof capable

of hybridising to one of <400>5 or <400>6 under low stringency conditions and which encodes an amino acid sequence corresponding to an amino acid sequence set forth in <400>7 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>7.

Yet another further aspect of the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in <400>5 or <400>6.

Still yet another further aspect of the present invention contemplates a the nucleotide sequence comprising a sequence of nucleotides as set forth in one of <400>1 or <400>3 or is a derivative, homologue or analogue thereof including a nucleotide sequence having similarity to one of <400>1 or <400>3 and which encodes an amino acid sequence corresponding to an amino acid sequence set forth in one of <400>2 or <400>4 or a sequence having at least about 45% similarity to 10 contiguous amino acids in one or more of <400>2 or <400>4.

Another further aspect of the present invention provides a nucleotide sequence comprising a sequence of nucleotides as set forth in one of <400>5 or <400>6 or is a derivative, homologue or analogue thereof including a nucleotide sequence having similarity to one of <400>5 or <400>6 and which encodes an amino acid sequence corresponding to an amino acid sequence as set forth in <400>7 or a sequence having at least about a 45% similarity to 10 contiguous amino acids in <400>7.

Another aspect of the present invention is directed to an isolated protein selected from the list consisting of:

(i) ELF5 wherein said ELF5 comprises an ETS domain or a derivative, homologue, analogue, chemical equivalent or mimetic thereof.

(ii) a protein having an amino acid sequence substantially as set forth in <400>2 or a derivative, homologue or mimetic thereof or a sequence having at least about 45%

similarity to at least 10 contiguous amino acids in <400>2 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

(iii) a protein having an amino acid sequence substantially as set forth in <400>4 or a derivative, homolog or mimetic thereof or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>4 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

(iv) a protein having an amino acid sequence substantially as set forth in <400>7 or a derivative, homologue or mimetic thereof or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>7 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

(v) a protein encoded by a nucleotide sequence substantially as set forth in <400>1 or a derivative, homologue or analogue thereof or a sequence encoding an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>2 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

(vi) a protein encoded by a nucleotide sequence substantially as set forth in <400>3 or a derivative, homologue or analogue thereof or a sequence encoding an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400> 4 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

(vii) a protein encoded by a nucleotide sequence substantially as set forth in <400>5 or <400>6 or a derivative, homologue or analogue thereof or a sequence encoding an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>7 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

(viii) a protein encoded by a nucleic acid molecule capable of hybridising to the nucleotide sequence as set forth in <400>1 or a derivative, homologue or analogue thereof under low stringency conditions and which encodes an amino acid sequence substantially as set forth in <400>2 or a derivative, homologue or mimetic thereof or an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>2.

(ix) a protein encoded by a nucleic acid molecule capable of hybridising to the nucleotide sequence as set forth in <400>3 or a derivative, homologue or analogue thereof under low stringency conditions and which encodes an amino acid sequence substantially as set forth in <400>4 or a derivative, homologue or mimetic thereof or an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>4.

(x) a protein encoded by a nucleic acid molecule capable of hybridising to the nucleotide sequence as set forth in <400>5 or <400>6 or a derivative, homologue or analogue thereof under low stringency conditions and which encodes an amino acid sequence substantially as set forth in <400>7 or a derivative, homologue or mimetic thereof or an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>6.

(xi) a protein as defined in any one of paragraphs (i) to (x) in a homodimeric form.

(xii) a protein as defined in any one of paragraphs (i) to (x) in a heterodimeric form.

Another aspect of the present invention contemplates a method of modulating activity of ELF5 in a mammal, said method comprising administering to said mammal a modulating effective amount of an agent for a time and under conditions sufficient to increase or decrease ELF5 activity.

Yet another aspect of the present invention contemplates a method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of a nucleotide sequence encoding *ELF5* or sufficient to modulate the activity of ELF5.

Still yet another aspect of the present invention contemplates a method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of ELF5 or *ELF5*.

A further aspect of the present invention relates to a method for the treatment and/or prophylaxis of a condition characterised by the aberrant, unwanted or otherwise inappropriate cellular functional activity, said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of *ELF5* or sufficient to modulate the activity of ELF5 wherein said modulation results in modulation of said cellular functional activity.

Yet another further aspect of the present invention relates to a method for the treatment and/or prophylaxis of a condition characterised by the aberrant, unwanted or otherwise inappropriate cellular functional activity, said method comprising administering to said mammal an effective amount of ELF5 or *ELF5* for a time and under conditions sufficient to modulate cellular functional activity.

Another aspect of the present invention provides a method for detecting an agent capable of modulating the function of ELF5 or its functional equivalent or derivative thereof said method comprising contacting a cell or extract thereof containing said ELF5 or its functional equivalent or derivative with a putative agent and detecting an altered expression phenotype associated with said ELF5 or its functional equivalent or derivative.

A further aspect of the present invention provides a method for detecting an agent capable of modulating the function of ELF5 or its functional equivalent or derivative thereof said

method comprising contacting an epithelial cell containing said ELF5 or its functional equivalent or derivative with a putative agent and detecting an altered proliferation rate.

In yet another aspect the present invention provides a method for detecting an agent capable of binding or otherwise associating with an ELF5 binding site or functional equivalent or derivative thereof said method comprising contacting a cell containing said ELF5 binding site or functional equivalent or derivative thereof with a putative agent and detecting an altered expression phenotype associated with modulation of the function of ELF5 or its functional equivalent or derivative.

Still yet another further aspect of the present invention relates to the use of an agent capable of modulating the expression of *ELF5* or modulating the activity of ELF5 in the manufacture of a medicament for the modulation of cellular functional activity.

Another aspect of the present invention relates to the use of ELF5 or *ELF5* in the manufacture of a medicament for the modulation of cellular functional activity.

Yet another aspect of the present invention relates to agents for use in modulating *ELF5* expression or ELF5 activity wherein said modulation results in modulation of cellular functional activity.

Still yet another aspect of the present invention relates to ELF5 or *ELF5* for use in modulating cellular functional activity.

A further aspect of the present invention contemplates a pharmaceutical composition comprising *ELF5*, ELF5 or an agent capable of modulating *ELF5* expression or ELF5 activity together with one or more pharmaceutically acceptable carriers and/or diluents. *ELF5*, ELF5 or said agent are referred to as the active ingredients.

Single and three letter abbreviations used throughout the specification are defined in Table 1.

TABLE 1

Single and three letter amino acid abbreviations

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of murine *ELF5* cDNA sequence and relationship to mRNA transcripts. (a) The nucleotide sequence of murine *ELF5* is shown. Breaks in the sequence indicate the source of sequence data; the central region (92-1528) was sequenced from lambda clones, and 5 prime and 3 prime were added from sequencing of RACE PCR products. Numbering of the nucleotides, starting with the most 5-prime sequences obtained, are indicated on the right. The open reading frame (ORF) is shown in capital letters, with the initiating start and stop codons underlined. A stop codon, in the same reading frame as the ORF, but 5 prime to the initiating codon, is also underlined. The ETS domain is indicated in a shaded box. Putative polyadenylation signals are underlined with dashed lines. A/T rich tracts in the 3 prime untranslated region are boxed. (b) Northern blot analysis of day 14 mouse placenta: lane 1, probed with random-prime-labeled 940 bp *Sty1* murine *ELF5* cDNA fragment (probe 1); lane 2, probed with random-prime-labeled murine *ELF5* 696 bp 3′-RACE PCR product (probe 2). Positions of 28S and 18S markers are indicated. Both lanes were also probed with GAPDH cDNA (lower panels).

Figure 2 is a schematic representation of (a) Comparison of human and mouse ORFs. Amino acid sequences present in both human and mouse ELF5 are shaded. The ETS domain is boxed with a solid line and the pointed domain with a dashed line. Putative phosphorylation sites, conserved between the two species are circled and labeled as CKII (casein kinase II), PKC (protein kinase C) or TyP (tyrosine kinase) substrates. (b) Comparison of the ETS domain of human and mouse ELF5 with those of known members of the ETS gene family. The alignment was generated using CLUSTAL W (Thompson *et al.*, 1994) with the default settings, and the result was subsequently adjusted manually. The ETS factors examined are labeled on the left and include hELF3, mELF3, hNERF, dETS4, dE74A, hELF1, hELK1, hTEL, hERM, mER81, mPEA3, mGABP∝ mERP, dETS6, mPU1, hPE1, hSAP1, hSPIB, dYAN, hERG, mFLI1, dELG, dETS3, mETS1, mETS2, mER71, where 'h' denotes human, 'm' mouse and 'd' *Drosophila*. The ETS consensus sequence is a list of the amino acids most often conserved between ETS family

members. Shading denotes amino acid identity with human ELF5, and the percent identity of each ETS domain is indicated on the right. (c) Phylogenetic tree of the ETS domain produced by maximum likelihood analysis. The alignment in Figure 2b was analysed using the JTT-F substitution model (Jones *et al.*, 1992) and local bootstrap values were estimated for all internal branches, both by using PROTML in Q mode followed by a second run in R mode (Adachi and Hasegawa, 1996). An underlying assumption of the phylogenetic analysis is that the amino acid content does not vary significantly among the sequences. This assumption was not assessed because tools for doing so are still under development. Therefore, the tree may be the result of both historical and compositional components. The four points at which gene duplications have been inferred are marked A, B, C and D. (d) Comparison of the pointed domain of human and mouse ELF5 with those of other members of the ETS family. The ETS factors examined are labeled on the left and include hERG, hELF3, hTEL, hGABP∝, hETS1, hETS2, dYAN and dPOINTEDP2. Other labels and conventions are as described for Figure 2b.

Figure 3 is a schematic representation of the chromosomal localization of human *ELF5*. Human chromosomal localization of ELF5 was performed by PCR using gene specific primers and the Genebridge 4 Radiation Hybrid DNA panel (UK HGMP Resource Centre). Diagram based upon PCR results showing localization of *ELF5* within chromosome 11, with respect to adjacent marker obtained from mapping data (see text).

Figure 4 is a photographic representation of *ELF5* expression in mouse tissues. Positions of 28S and 18S markers are indicated. *ELF5a* and *ELF5b* transcripts are indicated. (a) Northern analysis of adult mouse tissues probed with murine *ELF5* cDNA (top panels) and GAPDH cDNA (lower panels). Abbreviations; Li: liver; Lu: lung; Br: brain; Ki: kidney; He: heart; Sm: small intestine; Sp: spleen; Th: thymus; St: stomach; Ov: ovary; Pa: pancreas; To: tongue; Sk: skeletal muscle; Bl: bladder; 2Fa: day 2 pregnant fat; 2 Ma: day 2 pregnant mammary gland; 10 Fa: day 10 pregnant fat; 10 Ma: day 10 pregnant mammary gland; Co: colon. Arrow indicates position of brain specific transcript (see text). (b) Northern analysis as above, but using RNA from day 1 neonate mouse tissues. Additional abbreviation; In: intestine. Arrow indicates position of large transcript (see

text). (c) Northern analysis as above, but using RNA from day 16, 17 and 19 embryonic tissues. (d) Northern analysis as above, but using RNA from day 9.5 to day 19 placental tissues as indicated.

Figure 5 is a photographic representation of *ELF5* expression in human tissues and cell lines. (a) Northern analysis of adult human tissues probed with human *ELF5* cDNA (top panels) and β-Actin cDNA (lower panels). The single *ELF5* transcript is indicated. Other labels and conventions are as for Figure 4. Abbreviations; He: heart; Br: brain; Pl: placenta; Lu: lung; Li: liver; Sk: skeletal muscle; Ki: kidney; Pa: pancreas; Sp: spleen; Th: thymus; Pr: prostate; Te: testis; Ov: ovary; Sm: small intestine; Co: colon mucosa; PBL: peripheral blood lymphocytes. (b) RNAse protection analysis of *ELF5* and GAPDH in cell lines; 1: CaOv-3 (ovarian carcinoma); 2: BT-549 (ductal breast carcinoma); 3: ZR-75-1 (breast carcinoma); 4: T47D (ductal breast carcinoma, progesterone sensitive); 5: 786-O (renal adenocarcinoma); 6: SK-HEP-1 (liver adenocarcinoma); 7: A549 (lung adenocarcinoma); 8: CCL32SK (primary fibroblast); 9: MEL28 (melanoma); 10: WISH (amnion carcinoma); 11: Jurkat (T cell leukemia); 12: DU145 (prostate carcinoma); 13: PC3 (prostate carcinoma); 14: HEC-1 (endometrium carcinoma); 15: K562 (erythroid leukemia). (c) Southern analysis of *ELF5* in *Bgl*II digested genomic DNA from cell lines; 1: normal blood; 2: BT-549 (ductal breast carcinoma); 3: ZR-75-1 (breast carcinoma); 4: T47D (ductal breast carcinoma); 5: NCI-H1299 (large cell lung carcinoma); 6: NCI-HI87 (small cell lung carcinoma); 7: NCI-H322 (bronchioalveolar carcinoma); 8: NCI-H358 (bronchioalveolar carcinoma); 9: NCI-H522 (lung adenocarcinoma); 10: SK-LU-1 (lung adenocarcinoma); 11: NCI-H441 (bronchioalveolar carcinoma); 12: NCI-H460 (large cell lung carcinoma); 13: NCI-H661 (large cell lung carcinoma).

Figure 6 is a photographic representation of ELF5 binding to consensus ETS binding sequences. (a) His-tagged ELF5 recombinant protein, present in *E. coli* lysates (lane 2), was purified by metal-affinity chromatography to approximately 90% (lane 3) and eluted with imadazole (lane 4). (b) Specific DNA binding of Elf5 was analysed by electrophoretic mobility shift assay (EMSA), using labeled double-stranded oligonucleotides as probes. E74 contains a consensus binding site for ETS family

members (lane 1). E74ml is a mutant oligonucleotide based on E74, but with the core GGAA replaced by AGAA (lane 2). Binding to other consensus ETS sites was analysed by the ability of a 100-fold excess of unlabeled double-stranded oligonucleotide to compete with E74 for Elf5 binding GMETS contains an ETS binding site from the human GM-CSF promoter (lane 6). ERBB2 contains an ETS binding site from the human *erbB2*/HER2 promoter (lane 7). MSV contains an ETS binding site present in the long terminal repeat of the Moloney sarcoma virus (lane 8). AP1 contains a consensus AP1 binding site used as a negative control ELF5-DNA complexes are marked. Binding of ETS1 to E74 was used as a positive control (lane 10).

Figure 7 is a graphical representation of transactivation by ELF5. COS cells were co-transfected with CAT reporter and Elf5 expression constructs. Transcription of the CAT gene was driven by the thymidine kinase (tk) minimal promoter with five copies of the polyomavirus enhancer inserted upstream (p5Xpoly). The polyomavirus enhancer contains adjacent ETS and AP1 binding sites. The ELF5 sense construct (pBOSElf5as) was designed to express ELF5 protein, and the *ELF5* anti-s construct (pBOSElf5as) to produce anti-sense transcripts. In the absence of expression construct the equivalent amount of base vector (pEFBOS) was co-transfected. COS cells were processed for CAT assays and the results of at least four replicates are shown as the mean with standard error of the mean (s.e.m.) bars. Statistically significant results are indicated by asterisks. A single asterisk indicates moderate significance ($0.05 > P > 0.01$) and triple asterisks indicate very high significance ($P < 0.001$).

Figure 8 is a photographic representation of breast tissue sections from parafin-embedded samples which had been hybridized with ELF5 antisense RNA.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is predicated, in part, on the identification of a novel member of the ETS family of molecules, termed ELF5. The identification of this novel molecule permits the identification and rational design of a range of products for use in therapy, diagnosis and antibody generation involving, for example, regulation of cellular functional activity such as cellular proliferation. These therapeutic molecules may also act as either antagonists or agonists of ELF5 function and will be useful, *inter alia*, in cancer and autoimmune disease therapy.

Accordingly, one aspect of the present invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding ELF5 wherein said ELF5 comprises an ETS domain.

Reference to an "ETS domain" should be understood as a reference to a protein domain which recognises and binds to a purine rich GGA(A/T) motif of a promoter or enhancer (Macleod *et al.*, 1992; Wasylyk *et al.*, 1993; Janknecht and Nordheim, 1993; Werner *et al.*, 1995; Kodandapani *et al.*, 1996). The ETS domain may be continuous, meaning that it is comprised of a continuous sequence of amino acids, or it may be discontinuous, meaning that it is comprised of individual amino acids or sequences of amino acids from two or more separate regions of the protein and which are brought into proximity with one another to form the ETS domain due to the secondary, tertiary or quaternary structure of the protein.

More particularly, the present invention provides a nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>2 or <400>4 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to at least 10 contiguous amino acids in <400>2 or <400>4.

Another aspect of the present invention provides a nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>7 or a derivative, homologue or mimetic or having at least about 45% or greater similarity to at least 10 contiguous amino acids in <400>7.

The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid levels. Where there is non-identity of the nucleotide level "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. In a particular preferred embodiment, nucleotide and sequence comparisons are made at the level of identity rather than similarity. Any number of programs are available to compare nucleotide and amino acid sequences. Preferred programs have regard to an appropriate alignment. One such program is Gap which considers all possible alignment and gap positions and creates an alignment with the largest number of matched bases and the fewest gaps.. Gap uses the alignment method of Needleman and Wunsch. Gap reads a scoring matrix that contains values for every possible GCG symbol match. GAP is available on ANGIS (Australian National Genomic Information Service) at website http://mel1.angis.org.au..

Another aspect of the present invention contemplates a nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence substantially as set forth in <400>1 or <400>3 or a derivative or homologue thereof capable of hybridising to one of <400>1 or <400>3 under low stringency conditions and which encodes an amino acid sequence corresponding to an amino acid sequence set forth in one of <400>2 or <400>4 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in one of <400>2 or <400>4.

More particularly, the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in <400>1 or <400>3.

Another aspect of the present invention contemplates a nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence substantially as set forth in one of <400>5 or <400>6 or a derivative or homologue thereof capable of hybridising to one of <400>5 or <400>6 under low stringency conditions and which encodes an amino acid sequence corresponding to an amino acid sequence set forth in <400>7 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>7.

More particularly, the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in <400>5 or <400>6.

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions. In general, washing is carried out at T_m = 69.3 + 0.41 (G + C) % [19] = -12°C. However, the T_m of a duplex DNA decreases by 1°C with every increase of 1% in the number of mismatched based pairs (Banner *et al*, 1973).

The nucleic acid molecule according to this aspect of the present invention corresponds herein to "*ELF5*". This gene has been determined in accordance with the present invention to encode a protein which displays specific binding to DNA sequences

comprising a GGA(A/T) core. The product of the *ELF5* gene is referred to herein as ELF5. ELF5 is defined as belonging to the ETS family of transcription factors due to its expression of an ETS domain which recognises and binds the purine rich GGA(A/T) core motifs. ELF5 is a protein for which splice variants exist, thereby resulting in the expression of a variety of isoforms. Human ELF5 and human ELF5 short transcript are examples of 2 isoforms which differ in size due to the splicing out of exon regions from the *ELF5* mRNA molecule encoding the ELF5 short transcript. Murine *ELF5a* and *ELF5b* are examples of 2 mRNA transcripts which differ in the length of the 3' untranslated region. Human ELF5 and ELF5 short transcript are defined by the amino acid sequences set forth in <400>2 and <400>4, respectively and murine ELF5 is defined by the amino acid sequence set forth in <400>7. The cDNA nucleotide sequences for human *ELF5* and *ELF5* short transcript are defined by the nucleotide sequences set forth in <400>1 and <400>3, respectively, and murine *ELF5a* and *ELFb* are defined by the nucleotide sequences set forth in <400>5 and <400>6, respectively.

The nucleic acid molecules encoding ELF5 are preferably a sequence of deoxyribonucleic acids such as cDNA sequences or genomic sequences. A genomic sequence may also comprise exons and introns. A genomic sequence may also include a promoter region or other regulatory region.

Reference hereinafter to "ELF5" and "*ELF5*" should be understood as a reference to all forms of ELF5 and *ELF5*, respectively, including by way of example the two mRNA transcripts, *ELF5a* and *ELF5b*, observed in the mouse. Without limiting the invention in any way, sequence analysis of murine ELF5 has revealed two discrete polyadenylation signals present in the 3' untranslated region (UTR). The first of these (at 1391 bp) appears to be an overlapping poly(A)$^+$ recognition signal AATTAA and ATTAAAA. The second is a consensus polyadenylation signal, AATAAA, at 2181 bp. Sequence analysis of human *ELF5* has also revealed two mRNA transcripts arising from the splicing out of part of the exon region. Accordingly, the present invention should be understood to extend to all cDNA and peptide isoforms arising from alternative splicing of ELF5 mRNA. To the extent that it is not specified reference herein to "ELF5" and "*ELF5*"

should be understood to include reference to derivatives, homologues, analogues, chemical equivalents and mimetics thereof.

The protein and/or gene is preferably from a human, primate, livestock animal (eg. sheep, pig, cow, horse, donkey) laboratory test animal (eg. mouse, rat, rabbit, guinea pig) companion animal (eg. dog, cat), captive wild animal (eg. fox, kangaroo, deer), aves (eg. chicken, geese, duck, emu, ostrich), reptile or fish.

The term "protein" should be understood to encompass peptides, polypeptides and proteins. The protein may be glycosylated or unglycosylated, phosphorylated or dephosphorylated to various degrees and/or may contain a range of other molecules fused, linked, bound or otherwise associated to the protein such as amino acids, lipids, carbohydrates or other peptides, polypeptides or proteins. Reference hereinafer to a "protein" includes a protein comprising a sequence of amino acids as well as a protein associated with other molecules such as amino acids, lipids, carbohydrates or other peptides, polypeptides or proteins.

Derivatives include fragments, parts, portions, chemical equivalents, mutants, homologs, mimetics from natural, synthetic or recombinant sources including fusion proteins. Derivatives may be derived from insertion, deletion or substitution of amino acids. Amino acid insertional derivatives include amino and/or carboxylic terminal fusions as well as intrasequence insertions of single or multiple amino acids. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterized by the removal of one or more amino acids from the sequence. Substitutional amino acid variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. Additions to amino acid sequences including fusions with other peptides, polypeptides or proteins.

The derivatives of ELF5 include fragments having particular epitopes or parts of the entire ELF5 protein fused to peptides, polypeptides or other proteinaceous or non-proteinaceous molecules. For example, ELF5 or derivative thereof may be fused to a molecule to facilitate its entry into a cell. Analogs of ELF5 contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecules or their analogs. Derivatives of nucleic acid sequences may similarly be derived from single or multiple nucleotide substitutions, deletions and/or additions including fusion with other nucleic acid molecules. The derivatives of the nucleic acid molecules of the present invention include oligonucleotides, PCR primers, antisense molecules, molecules suitable for use in cosuppression and fusion of nucleic acid molecules.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with $NaBH_4$.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride

or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carboethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during protein synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid contemplated herein is shown in Table 2.

TABLE 2

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva
D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib

	D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
	D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
	D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
	D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
5	D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
	D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
	D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu
10	D-α-methylleucine	Dmleu	α-napthylalanine	Anap
	D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
	D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
	D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
15	D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
	D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
20	D-α-methylvaline	Dmval	N-cylcododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
25	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser
	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl))glycine	Nhis
30	D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen

N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomophenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L-α-methylleucine	Mleu	L-α-methyllysine	Mlys
L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
L-α-methylserine	Mser	L-α-methylthreonine	Mthr
L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr
L-α-methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane	Nmbc		

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer

groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety.

The nucleic acid molecule of the present invention is preferably in isolated form or ligated to a vector, such as an expression vector. By "isolated" is meant a nucleic acid molecule having undergone at least one purification step and this is conveniently defined, for example, by a composition comprising at least about 10% subject nucleic acid molecule, preferably at least about 20%, more preferably at least about 30%, still more preferably at least about 40-50%, even still more preferably at least about 60-70%, yet even still more preferably 80-90% or greater of subject nucleic acid molecule relative to other components as determined by molecular weight, encoding activity, nucleotide sequence, base composition or other convenient means. The nucleic acid molecule of the present invention may also be considered, in a preferred embodiment, to be biologically pure.

In a particularly preferred embodiment, the nucleotide sequence corresponding to *ELF5* is a cDNA sequence comprising a sequence of nucleotides as set forth in one of <400>1 or <400>3 or is a derivative, homologue or analogue thereof including a nucleotide sequence having similarity to one of <400>1 or <400>3 and which encodes an amino acid sequence corresponding to an amino acid sequence set forth in one of <400>2 or <400>4 or a sequence having at least about 45% similarity to 10 contiguous amino acids in one or more of <400>2 or <400>4.

In another particularly preferred embodiment, the nucleotide sequence corresponding to *ELF5* is a cDNA sequence comprising a sequence of nucleotides as set forth in one of <400>5 or <400>6 or is a derivative, homologue or analogue thereof including a nucleotide sequence having similarity to one of <400>5 or <400>6 and which encodes an amino acid sequence corresponding to an amino acid sequence as set forth in <400>7 or a sequence having at least about a 45% similarity to 10 contiguous amino acids in <400>7.

A derivative of the nucleic acid molecule of the present invention also includes nucleic acid molecules capable of hybridising to the nucleotide sequences as set forth in one of <400>1 or <400>3 or <400>5 or <400>6 under low stringency conditions. Preferably said low stringency is at 42°C.

The nucleic acid molecule may be ligated to an expression vector capable of expression in a prokaryotic cell (eg. *E. coli*) or a eukaryotic cell (eg. yeast cells, fungal cells, insect cells, mammalian cells or plant cells). The nucleic acid molecule may be ligated or fused or otherwise associated with a nucleic acid molecule encoding another entity such as, for example, a signal peptide, a cytokine or other member of the ETS family.

The expression product is ELF5 having an amino acid sequence set forth in one of <400>2 or <400>4 or <400>7 or is a derivative or homologue as hereinbefore defined or is a mammalian homologue having an amino acid sequence of at least about 45% similarity to at least 10 contiguous amino acids of the amino acid sequence set forth in one of <400>2 or <400>4 or <400>7.

Another aspect of the present invention is directed to an isolated protein selected from the list consisting of:

(i) ELF5 wherein said ELF5 comprises an ETS domain or a derivative, homologue, analogue, chemical equivalent or mimetic thereof.

(ii) a protein having an amino acid sequence substantially as set forth in <400>2 or a derivative, homologue or mimetic thereof or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>2 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

(iii) a protein having an amino acid sequence substantially as set forth in <400>4 or a derivative, homolog or mimetic thereof or a sequence having at least about 45%

similarity to at least 10 contiguous amino acids in <400>4 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

(iv) a protein having an amino acid sequence substantially as set forth in <400>7 or a derivative, homologue or mimetic thereof or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>7 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

(v) a protein encoded by a nucleotide sequence substantially as set forth in <400>1 or a derivative, homologue or analogue thereof or a sequence encoding an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>2 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

(vi) a protein encoded by a nucleotide sequence substantially as set forth in <400>3 or a derivative, homologue or analogue thereof or a sequence encoding an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400> 4 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

(vii) a protein encoded by a nucleotide sequence substantially as set forth in <400>5 or <400>6 or a derivative, homologue or analogue thereof or a sequence encoding an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>7 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

(viii) a protein encoded by a nucleic acid molecule capable of hybridising to the nucleotide sequence as set forth in <400>1 or a derivative, homologue or analogue thereof under low stringency conditions and which encodes an amino acid sequence substantially as set forth in <400>2 or a derivative, homologue or

mimetic thereof or an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>2.

(ix) a protein encoded by a nucleic acid molecule capable of hybridising to the nucleotide sequence as set forth in <400>3 or a derivative, homologue or analogue thereof under low stringency conditions and which encodes an amino acid sequence substantially as set forth in <400>4 or a derivative, homologue or mimetic thereof or an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>4.

(x) a protein encoded by a nucleic acid molecule capable of hybridising to the nucleotide sequence as set forth in <400>5 or <400>6 or a derivative, homologue or analogue thereof under low stringency conditions and which encodes an amino acid sequence substantially as set forth in <400>7 or a derivative, homologue or mimetic thereof or an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>6.

(xi) a protein as defined in any one of paragraphs (i) to (x) in a homodimeric form.

(xii) a protein as defined in any one of paragraphs (i) to (x) in a heterodimeric form.

The ELF5 of the present invention may be in multimeric form meaning that two or more molecules are associated together. Where the same ELF5 molecules are associated together, the complex is a homomultimer. An example of a homomultimer is a homodimer. Where at least one ELF5 is associated with at least one non-ELF5 molecule, then the complex is a heteromultimer such as a heterodimer. A heteromultimer may include a molecule of another member of the ETS family or other molecule capable of modulating transcription.

In accordance with the present invention, it is proposed that ELF5 is a molecule which regulates cellular functional activity. Reference to cellular "functional activity" should be

understood as a reference to the functions which a cell is capable of performing such as, but in no way limited to, one or more of proliferation, differentiation, cell surface molecule expression, antigen presentation, maintenance of viability, apoptosis, metabolism, signal transduction and molecular mechanisms such as transcription and translation. In one embodiment, the functional actvity is proliferation. Without limiting this invention to any one theory or mode of action, human *ELF5* has been mapped to human chromosome 11p13-15 which is a region that frequently undergoes loss of heterozygocity in several types of carcinoma, including breast, kidney and prostate carcinomas. The expression pattern of *ELF5* and ELF5 in normal and diseased tissues also supports a role for these molecules in the regulation of cellular functional activity and, in particular, in the direct or indirect regulation of tumorigenesis. Even more particularly, it is proposed that ELF5 functions as a transcription factor.

The cloning and sequencing of this gene and its expression product now provides an additional gene for use in the prophylactic and therapeutic treatment of diseases such as those involving aberrant cellular functional activity such as aberrant cellular proliferation. Examples of diseases involving aberrant cellular proliferation include diseases caused by excessive cellular proliferation, such as in tumorigenesis, or diseases caused by inadequate cellular proliferation. Accordingly, the present invention contemplates therapeutic and prophylactic uses of ELF5 amino acid and nucleic acid molecules, in addition to ELF5 agonistic and antagonistic agents, for the regulation of cellular functional activity, such as for example, regulation of proliferation, differentiation and/or regulation of gene expression by transcriptional regulation.

Still without limiting the present invention in any way, ELF5 maps to a chromosomal region known to harbour tumour suppressor genes. Further, its epithelial cell expression is lost in epithelial tumour cell lines and in the epithelial cells comprising primary breast carcinomas. Accordingly, in one embodiment of the present invention, the up-regulation of *ELF5* expression or ELF5 functional actvity in neoplastic epithelial cells is desired for the purpose of down-regulating cellular proliferation.

With respect to the modulation of cell proliferation, although the preferred method is to up-regulate ELF5 activity in order to inhibit or reduce neoplastic cell proliferation, the down-regulation of *ELF5* expression or ELF5 functional activity may also be desirable in certain circumstances. For example, to enhance cell proliferation in cellular senescence, ageing or tissue regeneration.

The present invention contemplates, therefore, a method for modulating expression of *ELF5* in a subject, said method comprising contacting *ELF5* gene with an effective amount of an agent for a time and under conditions sufficient to up-regulate or down-regulate or otherwise modulate expression of *ELF5*. For example, *ELF5* antisense sequences such as oligonucleotides may be introduced into a cell to up-regulate epithelial cell proliferative activity. Conversely, a nucleic acid molecule encoding ELF5 or a derivative thereof may be introduced to down-regulate proliferation of epithelial cells not expressing the endogenous *ELF5* gene.

The term "expression" refers to the transcription and translation of a nucleic acid molecule. Reference to "expression product" is a reference to the product produced from the transcription and translation of a nucleic acid molecule. Reference to "modulation" should be understood as a reference to up-regulation or down-regulation.

Another aspect of the present invention contemplates a method of modulating activity of ELF5 in a mammal, said method comprising administering to said mammal a modulating effective amount of an agent for a time and under conditions sufficient to increase or decrease ELF5 activity.

An "effective amount" means an amount necessary to at least partly attain the desired response.

Modulation of said activity by the administration of an agent to a mammal can be achieved by one of several techniques, including but in no way limited to introducing into said mammal a proteinaceous or non-proteinaceous molecule which:

(i) modulates expression of *ELF5*;

(ii) functions as an antagonist of ELF5;

(iii) functions as an agonist of ELF5 (including administration of ELF5 or functional equivalent, derivative, homologue, analogue or mimetic thereof).

Said proteinaceous molecule may be derived from natural or recombinant sources including fusion proteins or following, for example, natural product screening. Said non-proteinaceous molecule may be, for example, a nucleic acid molecule or may be derived from natural sources, such as for example natural product screening or may be chemically synthesised. The present invention contemplates chemical analogs of ELF5 capable of acting as agonists or antagonists of ELF5. Chemical agonists may not necessarily be derived from ELF5 but may share certain conformational similarities. Alternatively, chemical agonists may be specifically designed to mimic certain physiochemical properties of ELF5. Antagonists may be any compound capable of blocking, inhibiting or otherwise preventing ELF5 from carrying out its normal biological functions. Antagonists include monoclonal antibodies specific for ELF5, or parts of ELF5, and antisense nucleic acids which prevent transcription or translation of *ELF5* genes or mRNA in mammalian cells.

Said proteinaceous or non-proteinaceous molecule may act either directly or indirectly to modulate the expression of *ELF5* or the activity of ELF5. Said molecule acts directly if it associates with *ELF5* or ELF5 to modulate the expression or activity of *ELF5* or ELF5. Said molecule acts indirectly if it associates with a molecule other than *ELF5* or ELF5 which other molecule either directly or indirectly modulates the expression or activity of *ELF5* or ELF5. Accordingly, the method of the present invention encompasses the regulation of *ELF5* or ELF5 expression or activity via the induction of a cascade of regulatory steps which lead to the regulation of *ELF5* or ELF5 expression or activity.

Another aspect of the present invention contemplates a method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of a nucleotide sequence encoding *ELF5* or sufficient to modulate the activity of ELF5.

Preferably, said cellular functional activity is proliferation and even more preferably said modulation is down-regulation.

Yet another aspect of the present invention contemplates a method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of ELF5 or *ELF5*.

Preferably, said cellular functional activity is proliferation and even more preferably said modulation is down-regulation.

The ELF5, *ELF5* or agent used may also be linked to a targeting means such as a monoclonal antibody, which provides specific delivery of the ELF5, *ELF5* or agent to the target cells.

In a preferred embodiment of the present invention, the ELF5, *ELF5* or agent used in the method is linked to an antibody specific for said target cells to enable specific delivery to these cells.

Administration of the ELF5, *ELF5* or agent, in the form of a pharmaceutical composition, may be performed by any convenient means. ELF5, *ELF5* or agent of the pharmaceutical composition are contemplated to exhibit therapeutic activity when administered in an amount which depends on the particular case. The variation depends, for example, on the human or animal and the ELF5, *ELF5* or agent chosen. A broad range of doses may be applicable. Considering a patient, for example, from about 0.1 mg to about 1 mg of ELF5 or agent may be administered per kilogram of body weight

per day. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, weekly, monthly or other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of the situation. The ELF5 or agent may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intranasal, intraperitoneal, intramuscular, subcutaneous, intradermal or suppository routes or implanting (e.g. using slow release molecules). With particular reference to use of ELF5 or agent, these peptides may be administered in the form of pharmaceutically acceptable nontoxic salts, such as acid addition salts or metal complexes, e.g. with zinc, iron or the like (which are considered as salts for purposes of this application). Illustrative of such acid addition salts are hydrochloride, hydrobromide, sulphate, phosphate, maleate, acetate, citrate, benzoate, succinate, malate, ascorbate, tartrate and the like. If the active ingredient is to be administered in tablet form, the tablet may contain a binder such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid; and a lubricant, such as magnesium stearate.

A further aspect of the present invention relates to the use of the invention in relation to the therapeutic or prophylactic treatment of mammalian disease conditions involving aberrant, unwanted or otherwise inappropriate cellular functional activity. For example, the present invention is particularly useful, but in no way limited to, use in cancer therapy and in particular epithelial cancer therapy. For example, the method of the present invention may be used to treat epithelial tumours of the prostate, breast or lung.

Accordingly, another aspect of the present invention relates to a method for the treatment and/or prophylaxis of a condition characterised by aberrant, unwanted or otherwise inappropriate cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of *ELF5* or sufficient to modulate the activity of ELF5 wherein said modulation results in modulation of said cellular functional activity.

Preferably said condition is an epithelial cell tumour and said modulation is down-regulation of the proliferation of said epithelial cells. More preferably said tumour is malignant. Still more preferably said epithelial tumour is a tumour of the prostate, breast or lung.

In another aspect the present invention relates to a method for the treatment and/or prophylaxis of a condition characterised by aberrant, unwanted or otherwise inappropriate functional activity in a mammal said method comprising administering to said mammal an effective amount of ELF5 or *ELF5* for a time and under conditions sufficient to modulate said cellular functional activity.

Preferably said condition is an epithelial cell tumour and said modulation is down-regulation of the proliferation of said epithelial cells. More preferably said tumour is malignant. Still more preferably said epithelial tumour is a tumour of the prostate, breast or lung.

Reference to "aberrant, unwanted or otherwise inappropriate" cellular functional activity should be understood as a reference to over-activity, under-activity or to physiologically normal functional activity which is inappropriate in that it is unwanted or insufficient.

Yet another aspect of the present invention relates to the use of an agent capable of modulating the expression of *ELF5* or modulating the activity of ELF5 in the manufacture of a medicament for the modulation of cellular functional activity.

A further aspect of the present invention relates to the use of ELF5 or *ELF5* in the manufacture of a medicament for the modulation of cellular functional activity.

Still yet another aspect of the present invention relates to agents for use in modulating *ELF5* expression or ELF5 activity wherein said modulation results in modulation of cellular functional activity.

Another aspect of the present invention relates to ELF5 or *ELF5* for use in modulating cellular functional activity.

Administration of the agent, ELF5 or functional equivalent, derivative, homologue, analogue or mimetic thereof, or ELF5 nucleic acid molecule (herein referred to as "modulatory agent"), in the form of a pharmaceutical composition, may be performed by any convenient means. The modulatory agent of the pharmaceutical composition are contemplated to exhibit therapeutic activity when administered in an amount which depends on the particular case. The variation depends, for example, on the human or animal and the modulatory agent chosen. A broad range of doses may be applicable. Considering a patient, for example, from about 0.1 mg to about 1 mg of modulatory agent may be administered per kilogram of body weight per day. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, weekly, monthly or other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of the situation. The modulatory agent may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intraperitoneal, intramuscular, subcutaneous, intradermal or suppository routes or implanting (e.g. using slow release molecules). The modulatory agent may be administered in the form of pharmaceutically acceptable nontoxic salts, such as acid addition salts or metal complexes, e.g. with zinc, iron or the like (which are considered as salts for purposes of this application). Illustrative of such acid addition salts are hydrochloride, hydrobromide, sulphate, phosphate, maleate, acetate, citrate, benzoate, succinate, malate, ascorbate, tartrate and the like. If the active ingredient is to be administered in tablet form, the tablet may contain a binder such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid; and a lubricant, such as magnesium stearate.

In a related aspect of the present invention, the mammal undergoing treatment may be human or an animal in need of therapeutic or prophylactic treatment.

Reference herein to "treatment" and "prophylaxis" is to be considered in its broadest context. The term "treatment" does not necessarily imply that a mammal is treated until total recovery. Similarly, "prophylaxis" does not necessarily mean that the subject will not eventually contract a disease condition. Accordingly, treatment and prophylaxis include amelioration of the symptoms of a particular condition or preventing or otherwise reducing the risk of developing a particular condition. The term "prophylaxis" may be considered as reducing the severity of onset of a particular condition. "Treatment" may also reduce the severity of an existing condition or the frequency of acute attacks.

In accordance with these methods, the modulatory agent defined in accordance with the present invention may be coadministered with one or more other compounds or molecules. By "coadministered" is meant simultaneous administration in the same formulation or in two different formulations via the same or different routes or sequential administration by the same or different routes. By "sequential" administration is meant a time difference of from seconds, minutes, hours or days between the administration of the two types of molecules, These molecules may be administered in any order.

In yet another further aspect the present invention contemplates a pharmaceutical composition comprising *ELF5*, ELF5 or an agent capable of modulating *ELF5* expression or ELF5 activity together with one or more pharmaceutically acceptable carriers and/or diluents. *ELF5*, ELF5 or said agent are referred to as the active ingredients.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a

coating such as licithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thirmerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the

present invention are prepared so that an oral dosage unit form contains between about 0.1 μg and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to

produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 μg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 μg to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating *ELF5* expression or ELF5 activity. The vector may be, for example, a viral vector. The present invention should be understood to extend to the use of such vectors in gene therapy.

Screening for the modulatory agents hereinbefore defined can be achieved by any one of several suitable methods including, but in no way limited to, contacting a cell comprising the ELF5 gene or functional equivalent or derivative thereof with an agent and screening for the modulation of ELF5 protein production or functional activity, modulation of the expression of a nucleic acid molecule encoding ELF5 or modulation of the activity or expression of a downstream ELF5 cellular target. Detecting such modulation can be achieved utilising techniques such as Western blotting, electrophoretic mobility shift assays and/or the readout of reporters of ELF5 activity such as luciferases, CAT and the like.

It should be understood that the ELF5 gene or functional equivalent or derivative thereof may be naturally occurring in the cell which is the subject of testing or it may have been transfected into a host cell for the purpose of testing. Further, the naturally occurring or transfected gene may be constitutively expressed - thereby providing a model useful for, *inter alia*, screening for agents which down regulate ELF5 activity, at either the nucleic acid or expression product levels, or the gene may require activation - thereby providing a model useful for, *inter alia*, screening for agents which up regulate ELF5 expression. Further, to the extent that an ELF5 nucleic acid molecule is transfected into a cell, that molecule may comprise the entire ELF5 gene or it may merely comprise a portion of the gene such as the portion which regulates expression of the ELF5 product. For example, the ELF5 promoter region may be transfected into the cell which is the subject of testing. In this regard, where only the promoter is utilised, detecting modulation of the activity of the promoter can be achieved, for example, by ligating the promoter to a reporter gene. For example, the promoter may be ligated to luciferase or a CAT reporter, the modulation of expression of which gene can be detected via modulation of fluorescence intensity or CAT reporter activity, respectively.

In another example, the subject of detection could be a downstream ELF5 regulatory target, rather than ELF5 itself or the reporter molecule ligated to the ELF5 promoter or the reporter gene ligated to the promoter of the gene which ELF5 regulates. Yet another example includes ELF5 binding sites ligated to a minimal reporter. For example, modulation of ELF5 activity can be detected by screening for the modulation of the functional activity in an epithelial cell. This is an example of an indirect system where modulation of ELF5 expression, *per se*, is not the subject of detection. Rather, modulation of the molecules which ELF5 regulates the expression of, are monitored. Where the cell which is the subject of the screening system is an epithelial cell, modulation of ELF5 expression could be detected by screening for modulation of the proliferative activity of that cell.

These methods provide a mechanism for performing high throughput screening of putative modulatory agents such as the proteinaceous or non-proteinaceous agents

comprising synthetic, combinatorial, chemical and natural libraries. These methods will also facilitate the detection of agents which bind either the ELF5 nucleic acid molecule or expression product itself or which modulate the expression of an upstream molecule, which upstream molecule subsequently modulates ELF5 expression or expression product activity. Accordingly, these methods provide a mechanism of detecting agents which either directly or indirectly modulate ELF5 expression and/or activity.

Accordingly, another aspect of the present invention provides a method for detecting an agent capable of modulating the function of ELF5 or its functional equivalent or derivative thereof said method comprising contacting a cell or extract thereof containing said ELF5 or its functional equivalent or derivative with a putative agent and detecting an altered expression phenotype associated with said ELF5 or its functional equivalent or derivative.

Reference to "ELF5" should be understood as a reference to either the ELF5 expression product or a nucleic acid molecule encoding ELF5. It should also be understood as a reference to a portion or fragment of the ELF5 molecule such as the regulatory region of the ELF5 nucleic acid molecule. Alternatively, the molecule may comprise the binding portion of the ELF5 expression product. In this regard, the ELF5 nucleic acid molecule and/or expression product is expressed in a cell. The cell may be a host cell which has been transfected with the ELF5 nucleic acid molecule or it may be a cell, such as an epithelial cell, which naturally contains the ELF5 gene. Reference to "extraction thereof" should be understood as a reference to a cell free transcription system.

Reference to detecting an "altered expression phenotype associated with said ELF5" should be understood as the detection of cellular changes associated with modulation of the activity of ELF5. These may be detectable for example as intracellular changes or changes observable extracellularly. For example, this includes, but is not limited to, detecting changes in expression product levels, or, to the extent that the ELF5 regulatory region is ligated to a reporter molecule such as luciferase or CAT, detecting changes in reporter molecule expression. Alternatively, this screening system may be established to

detect changes in the expression of downstream molecules which are regulated by the ELF5 expression product. For example, detecting changes in mRNA or expression product levels of down-stream molecule or alteration to proliferation rates.

In a preferred embodiment, the present invention provides a method for detecting an agent capable of modulating the function of ELF5 or its functional equivalent or derivative thereof said method comprising contacting an epithelial cell containing said ELF5 or its functional equivalent or derivative with a putative agent and detecting an altered proliferation rate.

As detailed earlier, the method of this aspect of the present invention should be understood to extend to screening for agents which modulate the expression of ELF5 either directly or indirectly. An example of indirect modulation of ELF5 would be modulation of the expression of a first nucleic acid molecule, which first nucleic acid molecule expression product modulates the expression of a nucleic acid molecule encoding ELF5 or functional equivalent or derivative thereof.

In yet another aspect the present invention provides a method for detecting an agent capable of binding or otherwise associating with an ELF5 binding site or functional equivalent or derivative thereof said method comprising contacting a cell containing said ELF5 binding site or functional equivalent or derivative thereof with a putative agent and detecting an altered expression phenotype associated with modulation of the function of ELF5 or its functional equivalent or derivative.

Reference to "ELF5 binding site" should be understood as a reference to the nucleic acid sequence to which the ELF5 expression product can bind.

Still another aspect of the present invention is directed to antibodies to ELF5 including catalytic antibodies. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to ELF5 or may be specifically raised to ELF5. In the case of the latter, ELF5 may first need to be associated with a carrier

molecule. The antibodies and/or recombinant ELF5 of the present invention are particularly useful as therapeutic or diagnostic agents. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies. The antibodies of this aspect of the present invention are particularly useful for immunotherapy and may also be used as a diagnostic tool for assessing apoptosis or monitoring the program of a therapeutic regime.

For example, ELF5 can be used to screen for naturally occurring antibodies to ELF5.

For example, specific antibodies can be used to screen for ELF5 proteins. The latter would be important, for example, as a means for screening for levels of ELF5 in a cell extract or other biological fluid or purifying ELF5 made by recombinant means from culture supernatant fluid. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays, ELISA and flow cytometry.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of ELF5.

Both polyclonal and monoclonal antibodies are obtainable by immunization with the protein or peptide derivatives and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of ELF5, or antigenic parts thereof, collecting serum from the animal, and isolating specific sera by any of the known immunoadsorbent techniques. Although antibodies produced by this method are utilizable in virtually any

type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art. (See, for example Douillard and Hoffman, Basic Facts about Hybridomas, in *Compendium of Immunology* Vol II, ed. by Schwartz, 1981; Kohler and Milstein, *Nature 256*: 495-499, 1975; *European Journal of Immunology 6*: 511-519, 1976).

In another aspect of the present invention, the molecules of the present invention are also useful as screening targets for use in applications such as the diagnosis of disorders which are regulated by ELF5. For example, screening for the levels of ELF5 protein or *ELF5* mRNA transcripts in breast or prostate tissue as an indicator of a predisposition to, or the development of, breast or prostate cancer.

Yet another aspect of the present invention contemplates a method of diagnosing or monitoring a disease condition in a mammal, which disease condition is characterised by aberrant, unwanted or otherwise inappropriate cellular functional activity, said method comprising screening for ELF5 and/or *ELF5* in a biological sample isolated from said mammal.

Screening for ELF5 or *ELF5* in a biological sample can be performed by any one of a number of suitable methods which are well known to those skilled in the art. Examples of suitable methods include, but are not limited to, *in situ* hybridisation of biopsy sections to detect mRNA transcript or DNA, Northern blotting, RT-PCR of specimens isolated from tissue biopsies or bodily fluid samples (such as blood), antibody screening of tissue sections or bodily fluid samples.

To the extent that antibody based methods of diagnosis are used, the presence of ELF5 or *ELF5* may be determined in a number of ways such as by Western blotting, ELISA or flow cytometry procedures. These, of course, include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain ELF5 including cell extract, tissue biopsy or possibly serum, saliva, mucosal secretions, lymph, tissue fluid and respiratory fluid. The sample is, therefore, generally a biological sample comprising biological fluid but also extends to fermentation fluid and supernatant fluid such as from a cell culture.

In the typical forward sandwich assay, a first antibody having specificity for the ELF5 or antigenic parts thereof, is either covalently or passively bound to a solid surface. The

solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes) and under suitable conditions (e.g. 25°C) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the hapten. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Alternately, fluorescent compounds, such as fluorecein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic color visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescene and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

Further features of the present invention are more fully described in the following non-limiting examples.

SUMMARY OF SEQ ID Nos.

Sequence	SEQ ID NO.
nucleotide sequence of human ELF5	<400>1
amino acid sequence of human ELF5	<400>2
nucleotide sequence of human ELF5 short transcript	<400>3
amino acid sequence of human ELF5 short transcript	<400>4
nucleotide sequence of murine ELF5a	<400>5
nucleotide sequence of murine ELF5b	<400>6
amino acid sequence of murine ELF5	<400>7
oligonucleotide primer	<400>8
oligonucleotide primer	<400>9
oligonucleotide primer	<400>10
oligonucleotide primer	<400>11
oligonucleotide primer	<400>12
oligonucleotide primer	<400>13
oligonucleotide primer	<400>14
oligonucleotide primer	<400>15

EXAMPLE 1

ISOLATION OF MOUSE AND HUMAN ELF5 cDNAs

The murine ELF5 cDNA was isolated from an adult mouse lung cDNA library. Amalgamation of sequence data revealed a 1437 bp sequence with a maximum open reading frame (ORF) of 759 bp, predicted to encode a 253 amino acid protein of approximately 31 kD (Figure 1a). An upstream, in-frame ston codon suggests that this ORF represents the full-length coding sequence of ELF5. Additional 91 bp of 5', and 696 bp of 3' sequences were obtained by reverse transcriptase polymerase chain reaction (PCR) and rapid amplification of cDNA ends (RACE), using day 14 mouse placental RNA. Sequence analysis revealed two discrete polyadenylation signals present in the 3' untranslated region (UTR). The first of these (at 1391 bp) appears to be an overlapping poly(A)$^+$ recognition signal, AATTAA and ATTAAAA. The second is a consensus polyadenylation signal, AATAAA, at 2181 bp. These polyadenylation signals are found close to the 3' termination of the original clone and the 3' RACE product, respectively, suggesting that these represent polyA signals for two separate mRNA products. Thus, the two predicted ELF5 cDNAs are 2224 bp and 1528 bp long. Northern blot analysis, using the ELF5 coding sequence as a probe, confirmed the presence of two predominant ELF5 transcripts in placental tissue, ELF5a and ELF5b, of approximately 2.5 kb and 1.5 kb respectively. Only ELF5a was identified using a 3' UTR fragment from between the polyadenylation signals as a probe (Figure 1b), indicating that the transcripts differ in 3' UTR sequences.

A human ELF5 cDNA fragment was isolated from a human lung cDNA library following screening with a cDNA probe containing the coding sequence of mouse ELF5. The full coding sequence of human ELF5 was then obtained by reverse transcriptase PCR and RACE using human placental RNA. Analysis revealed that the ELF5 sequence is predicted to encode a 255 residue amino acid protein.

EXAMPLE 2

COMPARISON OF HUMAN AND MOUSE ELF5 AMINO ACID SEQUENCES

The predicted amino acid sequences of human and mouse ELF5 are highly conserved, with approximately 95% identity (Figure 2a). Only a single amino acid substitution was observed within the putative ETS domain of human and mouse ELF5, and most of the other differing amino acid residues in the full-length sequences are conservative substitutions (8/13), suggesting that the two proteins are homologs (i.e. having an inferred common ancestry). Interestingly, human ELF5 does, however, contain an additional two amino acid insertion compared to mouse ELF5. In addition to the ETS domain, other features appear to be conserved between these two sequences. These include a putative 'pointed' domain (Seth *et al.*, 1992; Lautenberger *et al.*, 1992) and several consensus casein kinase II (CKII) (Pinna, 1990), protein kinase C (PKC) (Kishimoto *et al.*, 1985; Woodget *et al.*, 1986) and tyrosine kinase (Patschinsky *et al.*, 1982; Hunter, 1982; Cooper *et al.*, 1984) phosphorylation sites.

The ETS domain found within all members of the ETS family is responsible for sequence-specific DNA binding (Seth *et al.*, 1992; Lautenberger *et al.*, 1992; Wasylyk *et al.*, 1993). The putative ETS domain of human/mouse ELF5, situated at the carboxyl terminal of the protein, is similar to that of human/mouse ELF3, with amino acid identity being 67%. However, this domain is only moderately similar to that of other ETS family members, with the highest amino acid identity being 49% to human NERF, 48% to *Drosophila* ETS4 and E74A, and 46% to human ELF1 and ELK1 (Figure 2b). Sequence identity to other family members is in the range of 44-36%. However, amino acids highly conserved amongst ETS family members (Janknecht and Nordheim, 1993) are well conserved in ELF5 (23/38). Some of these highly conserved residues, such as the three tryptophan residues in the carboxyl half of the ETS domain, have been demonstrated to be structurally critical for DNA binding of other ETS family members (Wang *et al.*, 1992; Wasylyk *et al.*, 1992).

Based on ETS domain similarities, a recent phylogenetic analysis (Graves and Petersen, 1998) has proposed the grouping of ETS factors into subfamilies, one of which is the ELF (E-74-like-factor) subfamily. The ELF subfamily includes *Drosophila* E74A, human ELF1 and NERF. A phylogenetic tree was generated including ELF5 and recently isolated ELF3, by maximum likelihood analysis of the ETS domain (Figure 2c). It shows that the human and mouse ELF5 sequences group most closely with the human and mouse ELF3 sequences, and that both ELF3 and ELF5 are most closely related to *Drosophila* ETS4, E74A and human ELF1 and NERF within the ETS family. Thus, *Drosophila* ETS4, and human/mouse ELF3 and ELF5 may also fall into the ELF subfamily of ETS factors.

The phylogency in Figure 2c shows the unrooted relationship among 28 ETS domains.

EXAMPLE 3

HUMAN CHROMOSOMAL MAPPING OF ELF5

Human chromosomal localization of ELF5 was performed by PCR, using gene specific primers and the Genebridge 4 Radiation Hybrid DNA panel (UK HGMP Resource Centre). With these primers, a single product of the expected size (234 bp) was amplified from total human DNA. The PCR reactions were then performed separately for each of the individual hybrids. The amplification results from the 93 hybrids were submitted to the Radiation Hybrid Mapping server at Whitehead Institute/MIT Center for Genome Research for analysis. The result demonstrated that ELF5 is localised to chromosome 11. The markers most tightly linked to ELF5 were D11S3990 (6.5cR) and D11S3998 (15.9 cR) (lod score $>$ 3.0), and these markers are located in the region of 11p13-15 (Figure 3). This chromosomal region frequently undergoes loss of heterozygosity (LOH) in several types of carcinoma (Baffa *et al.*, 1996; Dahiya *et al.*, 1997; Hirose *et al.*, 1996; Iizuka *et al.*, 1995; Kawana *et al.*, 1997; Lichy *et al.*, 1998; Wilson *et al.*, 1996).

EXAMPLE 4

EXPRESSION PATTERN OF ELF5 IN MOUSE TISSUES

Poly(A)$^+$ mRNA material derived from various mouse tissues were analysed by Northern blot hybridization using the murine ELF5 cDNA as a probe. A GAPDH probe was then used to control for RNA loading.

Analysis of ELF5 expression in adult mouse tissues revealed that ELF5 has a restricted expression pattern. Expression of two ELF5 transcripts, ELF5-a (2.5 kb) and ELF5-b (1.5 kb), were observed in lung (Lu), kidney (Ki), stomach (St), ovary (Ov), tongue (To), bladder (Bl), and day 2 pregnant (2 Ma) and day 10 pregnant (10 Ma) mammary glands, but no expression was observed in liver (Li), heart (He), small intestine (Sm), spleen (Sp), thymus (Th), pancreas (Pa), skeletal muscle (Sk), colon (Co) or fat (2 Fa and 10 Fa) (Figure 4a). Fat from day 2 (2 Fa) and day 10 (10 Fa) pregnant mice was used as a control for mammary expression, since the mammary gland contains much fat tissue. A single transcript was observed in brain (arrow - approximately 2.1 kb), but of a different size to either of the two ELF5 transcripts in other organs.

The expression of ELF5 was examined in the neonatal mouse (Figure 4b) and during embryogenesis on days 19, 17 and 16 (Figure 4c), and observed a similar expression pattern compared to that of the adult. However, at day 16 stage of embryogenesis low levels of ELF5 expression were detected in brain (regular sized transcripts) and small intestine, in addition to the expression pattern observed in the adult.

Placental expression of ELF5 displayed an interesting pattern during stages of embryogenesis (Figure 4d). Both transcripts were increasingly expressed from day 9.5 to day 13 before an overall decrease observed from day 14 to day 19, although some expression was observed at day 17.

The two predominant ELF5 mRNA transcripts were observed in variable ratios in different tissues, suggesting that polyadenylation sites may be utilized differentially, or

the two transcripts are subject to differential degradation. ELF5-a was expressed more strongly in neonatal and embryonic lung and kidney (Figures 4b and c), and adult ovary (Figure 4a), compared to ELF5-b. Conversely, ELF5-b was stronger in adult tongue (Figure 4a), and in all developmental stages of stomach (Figures 4a, b and c), compared to ELF5-a. In some RNA samples a further large (> 10 kb) transcript was variably observed.

EXAMPLE 5

EXPRESSION PATTERN OF ELF5 IN HUMAN TISSUES AND CANCER CELL LINES

Expression of ELF5 in adult human organs was also analysed by Northern blot of poly(A)$^+$ mRNA probed with the human ELF5 cDNA (Figure 5a). A single transcript of approximately 2.5 kb was strongly expressed in kidney (Ki) and prostate (Pr). However, much longer exposures of blots demonstrated just detectable expression of ELF5 in placenta (Pl) and lung (Lu). Further, ELF5 was cloned from human lung and placenta cDNA libraries, confirming that it is expressed in these tissues, albeit probably at very low levels.

ELF5 expression in human cancers was examined. A panel of cancer cell lines, including carcinomas of the ovary (CaOv-3), breast (BT-549, ZR-75-1, T47D), kidney (786-0), liver (SK-HEP-1), lung (A549), amnion (WISH), prostate (DU145, PC3) and endometrium (HEC-1), and melanoma (MEL28), T-cell leukemia (Jurkat) and erythroid leukemia (K562), were analysed for ELF5 expression by RNAse protection assay (Figure 5b). A primary fibroblast cell line (CCL32SK) was also included as a sample of non-transformed cells. Of all these cell lines only T47D, a progesterone sensitive ductal breast carcinoma, was observed to express ELF5.

To evaluate the possibility that lack of ELF5 expression in carcinoma was due to genomic alterations, a panel of breast and lung carcinoma derived cell lines were analysed by Southern blot (Figure 5c). ELF5 gene dosage was compared to that present in DNA

from normal human blood (based on the 6.5 kb *Bgl*II fragment) and controlled by hybridization with a β-*actin* cDNA probe. These results are summarized in the lower panel, where '2' represents a normal allele complement. No evidence was found for allelic loss or gene rearrangement in the two breast carcinoma cell lines that did not express ELF5 (BT-549 - lane 2, ZR-75-1 - lane 3). However, of nine lung carcinoma cell lines, evidence for loss of an ELF5 allele was observed in two (NCI-H358 - lane 8, NCI-H441 - lane 11). Hybridization with an ELF3 cDNA probe, which is localised to the long arm of chromosome 1 (Tymms *et al.*, 1997), helped to confirm the specific loss of ELF5 alleles. Two other lung carcinoma lines (SK-LU-1 - lane 10, NCI-H661 - lane 13) displayed hybridization with multiple fragments (shaded arrows) in addition to those observed in normal DNA (solid arrows), possibly indicating that at least one ELF5 allele has been rearranged in these lines. Confirmation of rearrangement, rather than restriction fragment length polymorphism (RFLP), was made by additional restriction digests. Some cell lines appeared to have amplification or additional copies of the ELF5 gene. One of these, T47D (lane 4), was the only cell line demonstrated to express ELF5, and another, SK-LU-1 (lane 10), appeared to have rearranged alleles.

EXAMPLE 6

SEQUENCE-SPECIFIC BINDING OF ELF5 TO DNA SEQUENCES CONTAINING CONSENSUS ETS SITES

Although ELF5 displays similarity to the consensus ETS domain, characterising it as an ETS family member, this sequence is still quite divergent from most other ETS family members. The hallmark of ETS factors to bind DNA sites containing a GGAA-core in a sequence-specific manner is however shared by ELF5, demonstrating an additional functional similarity to the ETS family. A recombinant ELF5 HIS-tag protein of approximately 29 kD, expressed in *E. coli* and purified by metal-affinity chromatography (Figure 6a, lane 4), displayed strong binding to consensus ETS binding sites, as analysed by electrophoretic mobility shift assay (EMSA) (Figure 6b). ELF5 bound the E74 oligonucleotide (containing a GGAA-core) (lane 1), but not to the E74ml oligonucleotide (which had been mutated to an AGAA-core) (lane 2). The first G-residue of the core has

been demonstrated to be a physical point of DNA contact for ETS1, and consequently essential for DNA binding (Fisher *et al.*, 1991; Nye *et al.*, 1992). Thus, ELF5 displays sequence specific binding to a consensus ETS binding site, binding that is disrupted by a mutation known to similarly affect other ETS family members. These results were confirmed through competition analysis. The ELF5-E74 complex (lane 3) was efficiently competed by the addition of a 100-fold excess of unlabeled E74 (lane 4), but not by E74ml (lane 5).

ELF5 also displayed sequence specific binding to different consensus ETS binding sequences, and did so with differential affinity (Figure 6b). Competition of the ELF5-E74 complex (lane 3) was achieved by consensus ETS sites from the GM-CSF promoter (lane 6), *erb*-B2 promoter (lane 7) and moloney sarcoma virus (MSV) long terminal repeat (LTR) (lane 8). The relative ability of ELF5 to bind these sequences occurred in the order: E74 > erbB2 > MSV > GM-CSF. ELF5 did not appear to be competed at all by an oligonucleotide containing a consensus AP1 binding site (lane 9). ETS1 binding to E74 was used as a positive control (lane 10).

EXAMPLE 7

MOUSE ELF5 ACTS AS A TRANSCRIPTIONAL ACTIVATOR

In addition to DNA binding, another characteristic of most ETS factors is their ability to transactivate from binding sites in promoters and enhancers.

A reporter construct, containing the chloramphenicol acetyl-transferase (CAT) driven by a minimal TK promoter and multiple ETS/AP1 binding sites (from the polyomavirus enhancer), was co-transfected into COS cells together with an ELF5 expression construct (Figure 7). Analysis of CAT activities revealed that ELF5 expression resulted in an average five-fold transactivation of the reporter. Further, this transactivation was inhibited by addition of an anti-sense ELF5 mRNA expression vector, indicating that ELF5 transactivation was due specifically to the product translated from the sense construct.

EXAMPLE 8

ISOLATION AND CHARACTERIZATION OF FULL-LENGTH MURINE ELF5 CDNA

The murine *Elf5* cDNA was isolated from an adult lung cDNA library in Lambda ZAPII (Stratagene) following screening with a cDNA probe containing the ETS domain region of human *ELF3*. Additional 5' sequence and 3' sequence were obtained by RT-PCR using a Marathon cDNA synthesis Kit (Clontech) and RACE (Rapid Amplification using day 14 murine of cDNA Ends) placental Poly(A)$^-$ RNA. The murine *Elf5*-specific PCR products were cloned into pGEM-T vector (Promega Corp., Madison, WI, USA). All cDNA sequences were confirmed by sequencing both strands at least once. 5'RACE gene-specific primer 1:5'GCCAGTCTTG-GTCTCTTCAGCATC-3' (<400>8); 5'-RACE nested-gene-specific primer 2: 5'-AGGAGATGCAGTTGGCATCAAGCT-3' (<400>9); 3'-RACE gene-specific primer 1: 5'-AGCCAGTGTTATGGGTGCTG-3' (<400>10); 3'-RACE nested-gene-specific primer 2: 5'-ACAGTCACTTGATCCACGGCCAATCC-3' (<400>11).

EXAMPLE 9

ISOLATION OF HUMAN ELF5 CODING SEQUENCE

A human *ELF5* cDNA fragment was isolated from a human lung cDNA library (GIBCO BRL) following screening with a cDNA probe containing the coding sequence of mouse *Elf5*. The coding sequence was then obtained by RT-PCR using a Marathon cDNA synthesis Kit (Clontech) and RACE (Rapid Amplification of cDNA Ends) using human placental Poly(A)$^+$ RNA. The human *ELF5*-specific PCR products were cloned into pGEM-T vector (Promega Corp., Madison, WI, USA). All cDNA sequences were confirmed by sequencing both strands at least once.

EXAMPLE 10
STS CONTENT MAPPING

The following sequence specific primers for human ELF5 were used for PCR. Forward primer: 5'-CCTGTGACTCATACTGGACATC-3' (<400>12); Reverse primer: 5'-CTTGTGTGCGGATGTTCTGG-3' (<400>13). The PCR reactions were performed in Opti-Primer™ 10 x buffer #3 (100 mM Tris-HCl pH 8.3, 35 mM $MgCl_2$, 250 mM Kcl) with 1 μl of Master Mix 50 x buffer (20 mM Tris-HCl pH 8.0, 250 nM EDTA) (Opti-Primer™ PCR Optimization Kit, Stratagene), 50 ng of template DNA, 0.2 μg of each primer, 1 μl of 10 mM dNTPs and 0.25 U of Taq DNA polymerase in a total volume of 50 μl. PCR parameters were an initial denaturation step at 94°C for 1 min, followed by 30 cycles of 94°C (1 min), 60°C (1 min), 72°C (1 min). For Genebridge 4 Radiation Hybrid DNA panel (UK GHMP Resource Centre), PCR reactions were performed separately for each of the individual hybrids. The PCR results from the 93 hybrids were submitted to the Radiation Hybrid Mapping server at Whitehead Institute/MIT Center for Genome Research (http://www.genome.wi.mit.edu/cgibin/contig/rhmapper.pl). The STS content mapping experiment was performed in duplicate and included PCR reactions with no DNA, total human DNA and total hamster DNA as controls.

EXAMPLE 11
SOUTHERN AND NORTHERN BLOT ANALYSIS

Northern analysis of *ELF5* expression in human adult organs was performed with commercially available blots containing 2 μg of Poly(A)$^+$ RNA (Clontech). For other Northern blots POLY(A)$^+$ mRNA was isolated by a modification of Gonda *et al.* (1992). Genomic DNA was isolated by standard techniques (Sambrook *et al*, 1997). Random-primed probes using a 898 bp human *ELF5* cDNA fragment and a 940 bp *StyI* mouse *Elf5* cDNA fragment were generated and Southern/Northern hybridizations performed using standard procedures. Blots were re-probed with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or β-*actin* cDNAs to verify RNA/DNA loading.

EXAMPLE 12

RNASE PROTECTION ANALYSIS

ELF5 mRNA abundance in total RNA from human cell lines was determined as described previously (Tymms, 1995). Anti-sense RNA probes for human *ELF5* and GAPDH transcribed from linearized plasmid vectors generated full-length probes of 388 bp and 216 bp, respectively. The protected products generated by hybridization and RNAse digestion are 298 bp for *ELF5* and 150 bp for GAPDH.

EXAMPLE 13

CELL LINES AND CULTURE

Monkey COS7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin, and maintained in a humidified incubator at 5% CO_2 and 37°C.

EXAMPLE 14

PLASMIDS

pHis6-Elf5 expression vector was made as follows: The murine *Elf5* cDNA was amplified using PCR oligonucleotide primers (5'-CGGGATCCTTGGACTCCGTAACCCATAGC-3' (<400>14) and 5'-GCAGATCTCAGAGTTTCTCTTCCTGCC-3' (<400>15)) containing a *Bam*HI restriction site followed by 21 nucleotides of the murine *Elf5* coding sequence and a *Bgl*II restriction site followed by 19 nucleotides complementary to the last 20 nucleotides of the *Elf5* coding sequence. The PCR fragment was cloned into the pGEM-T vector (Promega Corp., Madison, WI, USA), the *Bam*HI-*Sac*I restriction fragment with the *Elf5* coding sequence was then cloned into the *Bam*HI-*Sac*I sites of the pQE30 (Qiagen, Inc. Chatsworth, CA, USA) bacterial expression vector resulting in a N-terminal fusion of *Elf5* protein to six histidine residues (His-Tag).

The *Elf5* mammalian expression construct (pBOSElf5s) contains the full mouse *Elf5* cDNA blunt cloned into the T4 polymerase blunted *Xba*I site of pEFBOS (Mizushima and Natata, 1990). Expression from pEFBOS is driven by the elongation factor-1 promoer. The *Elf5* anti-sense expression construct is similar, but with reverse orientation of the *Elf5* polyomavirus enhancer oligonucleotides into the *Bam*HI site of pBLCAT2.

EXAMPLE 15

ELECTROPHORETIC MOBILITY SHIFT ASSAYS (EMSA)

Purified recombinant *Elf5* and Ets1 proteins were produced as 6XHis-tag fusions in *E. coli* using the QIAexpress expression system (Qiagen). Overnight cultures were diluted 1/10 in LB broth and grown for 1 h at 37°C. Expression of recombinant proteins were induced by addition of 0.1 mM IPTG and culture of cells for 2 h. Cells were harvested and sonicated in lysis buffer (6 M guanidine, 20 mM Tris-HCl, 50 mM NaCl, pH 8.0), and cell debris removed by centrifugation. One ml of metal His-affinity resin was incubated with supernatants for 30 min, collected, washed in wash buffer (8 M urea, 20 mM Tris-HCl, 50 mM NaCl, pH 8.0), and resuspended in renaturation buffer (20 mM Tris-HCl, 50 mM NaCl, 3 mM dithiothreitol (DTT), pH 8.0). Proteins were eluted from the beads in renaturation buffer supplemented with 100 mM imidazole. Purification and integrity of recombinant proteins were confirmed by denaturing SDS-polyacrylamide gel electrophoresis (PAGE).

DNA binding experiments with recombinant proteins were performed using EMSA, as previously described (Thomas *et al*, 1995, 1997). Briefly, purified double stranded oligonucleotides were labeled with γ-^{32}P dATP and T4 polynucleotide kinase. Oligonucleotide probe (1 ng) was incubated for 10 min with approximately 20 ng purified *Elf5/Ets1* protein in DNA binding buffer (1 mM EDTA, 10 mM Tris-Hcl pH 8.0, 50 mM NaCl, 3 mM DTT, 1 mg/ml BSA, 500 ng/ml poly-d(I-C)d(I-C), 500 ng/ml poly dI-dC, 200 ng/ml sheared salmon sperm DNA), $\pm$ 100 ng unlabelled competitor oligonucleotides, in 10 μl final volume. Assays were run through non-denaturing, 7% acrylamide (29 acrylamide:1 bis-acrylamide), 0.5 x TBE gels at 4°C.

EXAMPLE 16

LACK OF ELF5 EXPRESSION IN HUMAN PRIMARY BREAST CARCINOMAS

A panel of human primary breast carcinoma samples were analysed for ELF5 expression by *in situ* hybridization (Hogan *et al.*, 1994). Section from parafin-embedded samples were hybridized with ELF5 ^{33}P-labelled antisense RNA, and signals were detected with a photosensitive emulsion. Serial sections were also stained with eosin and haematoxylin.

A preliminary examination of ELF5 expression shows that ELF5 is not detectable in 20 out of 20 human breast carcinomas studied, whereas it is strongly expressed in adjacent normal epithelium and in epithelial cells from normal subjects (Figure 8).

EXAMPLE 17

REGULATION OF CELLULAR PROLIFERATION BY *ELF5*

The human cancer cell lines PC-3 (prostate cancer cell line) and MDA-MB-435 (breast ductal carcinoma cell line) were transfected with either human or murine ELF5 cDNA under the BOS promoter. Neither of these cell lines expresses ELF5 natively. ELF5 transfected clones grew slower, showed greater death and showed an altered morphology compared with empty vector transfected control cells.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

BIBLIOGRAPHY

Adachi J and Hasegawa M., *MOLPHY Version 2.3: Programs for molecular phylogenetics based on maximum likelihood.* Computer Science Monographs. The Institute of Statistical Mathematics, Tokyo

Baffa R, Negrini M, Mandes B, Rugge M, Ranzani GN, Hirohashi S and Croce CM., *Cancer Res.*, **56**:268-272 (1996)

Ben-David Y., Giddens EB., Letwin K and Bernstein A., *Genes. Dev.*, **5**:908-918 (1991)

Banner *et al.*, J. Mol. Biol., **81**:173 (1973)

Dahiya R, McCarville J, Lee C, Hu W, Kaur G, Carroll P and Deng G., *Int. J. Cancer.*, **72**:283-288 (1997)

Bhat NK, Komschlies KL, Fujiwara S, Fisher RJ, Mathieson BJ, Gregorio TA, Young HA, Kasik JW, Ozato K and Papas TS., *J. Immunol.*, **142**:672-678 (1989)

Bhat NK, Thompson CB, Lindsten T, June CH, Fujiwara S, Koizumi S, Fisher RJ and Papas TS., *Proc. Natl. Acad. Sci. USA.*, **87**:3723-3727 (1990)

Buttice G and Kurkinen M., *J. Biol. Chem.*, **268**:7196-7204 (1993)

Buttice G, Duterque-Coquillaud M, Basuyaux JP, Carrere S, Kurkinen M and Stehelin D., *Oncogene.*, **13**:2297-2306 (1996)

Chang CH, Scott GK, Kuo WL, Xiong X, Suzdaltseva Y, Park JW, Sayre P, Erny K, Collins C, Gray JW and Benz CC., *Oncogene.*, **14**:1617-1622 (1997)

Choi SG, Yi Y, Kim YS, Kato M, Chang J, Chung HW, Hahm KB, Yang HK, Rhee HH, Bang YJ and Kim SJ., *J. Biol. Chem.*, **273**:110-117 (1998)

Cooper JA, Esch FS, Taylor SS and Hunter T., *J. Biol. Chem.*, **259**:7835-7841 (1984)

Delannoy-Courdent A, Fauquette W, Dong-Le Bourhis XF, Boilly B, Vandenbunder B and Desbiens X., *Int. J. Dev. Biol.*, **40**:1097-1108 (1996)

Fisher RJ, Mavrothalassitis G, Kondoh A and Papas TS., *Oncogene.*, **6**:2249-2254 (1991)

Golub TR, Barker GF, Bohlander SK, Hiebert SW, Ward DC, Bray-Ward P, Morgan E, Raimondi SC, Rowley JD and Gilliland DG., *Proc. Natl, Acad. Sci. USA.*, **92**:4917-4921 (1995)

Golub TR, Barker GF, Lovett M and Gilliland DG., *Cell.*, **77**:307-316 (1994)

Gonda TJ, Sheiness DK and Bishop JM., *Mol. Cell Biol.*, **2**:617-624 (1992)

Graves BL and Petersen JM., *Adv. Cancer Res.*, in press (1998)

Gutman A and Wasylyk B., *Trends Genet.*, **7**:49-54 (1991)

Hart AH, Corrick CM, Tymms MJ, Hertzog PJ and Kola I., *Oncogene.*, **10**:1423-1430 (1995)

Hirose M, Yamada T, Toyosaka A, Hirose T, Kagami S, Abe T and Kuroda Y., *Med. Pediatr. Oncol.*, **27**:174-178 (1996)

Hogan B, Beddington R, Costantini F and Lacy E., *A Laboratory Manual. 2nd edn. Cold Spring Harbor Laboratory Press.*, **p**.344-351 (1994)

Hunter T., *J. Biol. Chem.*, **257**:4843-4848 (1982)

Ida K, Kobayashi S, Taki T, Hanada R, Bessho F, Yamamori S, Sugimoto T, Ohki M and Hayashi Y., *Int. J. Cancer.*, **63**:500-504 (1995)

Iizuka M, Sugiyama Y, Shiraishi M, Jones C and Sekiya T., *Genes Chromosomes Cancer.*, **13**:40-46 (1995)

Janknecht R and Nordheim A., *Biochim. Biophys. Acta.*, **1155**:346-356 (1993)

Jones DT, Taylor WR and Thornton JM., *Nature.*, **358**:86-89 (1992)

Karim FD, Urness LD, Thummel CS, Klemsz MJ, McKercher SR, Celada A, Van Beveren C, Maki RA, Gunther CV, Nye JA and Graves BJ., *Genes Dev.*, **4**:1451-1453 (1990)

Kawana Y, Komiya A, Ueda T, Nihei N, Kuramochi H, Suzuki H, Yatani R, Imai T, Dong JT, Imai T, Yoshie O, Barrett JC, Isaacs JT, Shimazaki J, Ito H and Ichikawa T., *Prostate.*, **32**:205-213 (1997)

Kishimoto A, Nishiyama K, Nakanishi H, Uratsuji Y, Nomura H, Takeyama Y and Nishizuka Y., *J. Biol. Chem.*, **260**:12492-12499 (1985)

Kodandapani R, Pio F, Ni CZ, Piccialli G, Klemsz M, McKercher S, Maki RA and Ely KR., *Nature.*, **380**:456-460 (1996)

Kola I, Brookes S, Green AR, Garber R, Tymms M, Papas TS and Seth A., *Proc. Natl. Acad. Sci. USA.*, **90**:7588-7592 (1993)

Lautenberger JA, Burdett LA, Gunnell MA, Qi S, Watson DK, O'Brien SJ and Papas TS., *Oncogene.*, **7**:1713-1719 (1992)

Lichy JH, Zavar M, Tsai MM, O'Leary TJ and Taubenberger JK., *Am. J. Pathol.*, **153**:271-278 (1998)

Macleod K, Leprince D and Stehelin D., *Trends Biochem. Sci.*, **17**:251-256 (1992)

Mizushima S and Nagata S., *Nucleic Acids Res.*, **18**:5322 (1990)

McKercher SR, Torbett BE, Anderson KL, Henkel GW, Vestal DJ, Baribault H, Klemsz M, Feeney AJ, Wu GE, Paige CJ and Maki RA., *EMBO J.*, **15**:5647-5658 (1996)

Moreau-Gachelin F, Wendling F, Molina T, Denis N, Titeux M, Grimber G, Briand P, Vainchenker W and Tavitian A., *Mol. Cell Biol.*, **16**:2453-2463 (1996)

Muthusamy N, Barton K and Leiden JM., *Nature.*, **377**:639-642 (1995)

Nunn MF, Seeburg PH, Moscovici C and Duesberg PH., *Nature.*, **306**:391-395 (1983)

Nye JA, Petersen JM, Gunther CV, Jonsen MD and Graves BJ., *Genes Dev.*, **6**:975-990 (1992)

O'Neill EM, Rebay I, Tjian R and Rubin GM., *Cell.*, **78**:137-147 (1994)

Oettgen P, Alani RM, Barcinski MA, Brown L, Akbarali Y, Boltax J, Kunsch C, Munger K and Libermann TA., *Mol. Cell Biol.*, 17:4419-4433 (1997)

Patschinsky T, Hunter T, Esch FS, Cooper JA and Sefton BM., *Proc. Natl. Acad. Sci. USA.*, **79**:973-977 (1982)

Peeters P, Raynaud SD, Cools J, Wlodarska I, Grosgeorge J, Philip P, Monpoux F, Van Rompaey L, Baens M, Van den Berghe H and Marynen P., *Blood.*, **90**:2535-2540 (1997)

Peter M, Couturier J, Pacquement H, Michon J, Thomas G, Magdelenat H and Delattre O., *Oncogene.*, **14**:1159-1164 (1997)

Pinna LA., *Biochim. Biophys. Acta.*, **1054**:267-284 (1990)

Sambrook J, Fritsch ER and Maniatis T., *Molecular Cloning: A Laboratory Manual 2nd edn. Cold Spring Harbor Laboratory Press.*, (1997)

Scott EW, Simon MC, Anastasi J and Singh H., *Science.*, **265**:1573-1577 (1994a)

Scott GK, Daniel JC, Xiong X, Maki RA, Kabat D and Benz CC., *J. Biol. Chem.*, **269**:19848-19858 (1994b)

Seth A and Papas TS., *Oncogene.*, **5**:1761-1767 (1990)

Seth A, Ascione R, Fisher RJ, Mavrothalassitis GJ, Bhat NK and Papas TS., **3**:327-334 (1992)

Seth A, Watson DK, Blair DG and Papas TS., *Proc. Natl. Acad. Sci. USA.*, **86**:7833-7837 (1989)

Sorensen PH, Lessnick SL, Lopez-Terrada D, Liu XF, Triche TJ and Denny CT., *Nat. Genet.*, **6**:146-151 (1994)

Sumarsono SH, Wilson TJ, Tymms MJ, Venter DJ, Corrick CM, Kola R, Lahoud MH, Papas TS, Seth A and Kola I., *Nature.*, **379**:534-537 (1996)

Sun W, Graves BJ and Speck NA., *J. Virol.*, **69**:4941-4949 (1995)

Thomas RS, Tymms MJ, Mckinlay LH, Shannon MF, Seth A and Kola I., *Oncogene*, **14**:2845-2855 (1997)

Thomas RS, Tymms MJ, Seth A, Shannon MF and Kola I., *Oncogene*, **11**:2135-2143 (1995)

Thompson, JD, Higgins, DG and Gibson, TJ *Nucleic Acids Research* **22**:4673-4680 (1994)

Tymms MJ, *Methods Mol. Biol.*, **37**:31-46 (1995)

Tymms MJ, Ng AY, Thomas RS, Schutte BC, Zhou J, Eyre HJ, Sutherland GR, Seth A, Rosenberg M, Papas T, Debouck C and Kola I, *Oncogene,* **15**:2449-2462 (1997)

Vandenbunder B, Wenert N, Queva C, Desbiens X and Stehelin D, *Folia. Biol. (Praha.)*, **40**:301-313 (1994)

Wang CY, Petryniak B, Ho IC, Thompson CB and Leiden JM, *J. Exp. Med.*, **175**:1391-1399 (1992)

Wasylyk B, Hahn SL and Giovane A, *Eur. J. Biochem.*, **211**:-7-18 (1993)

Wasylyk C, Gutman A, Nicholson R, and Wasylyk B, *EMBO. J.*, **10**:1127-1134 (1991)

Wasylyk C, Kerckaert JP and Wasylyk B, *Genes. Dev.*, **6**:965-974 (1992)

Watson DK, McWilliams MJ, Lapis P, Lautenberger JA, Schweinfest CW and Papas TS, *Proc. Natl. Acad. Sci. USA,* **85**:7862-7866 (1988)

Werner MH, Clore M, Fisher CL, Fisher RJ, Trinh L, Shiloach J and Gronenborn AM, *Cell*, **83**:761-771 (1995)

Wernert N, Raes MB, Lassalle P, Dehouck MP, Gosselin B, Vandenbunder B and Stehelin D. *Am. J. Pathol.*, **140**:119-127 (1992)

Wilson AP, Dent M, Pejovic T, Hubbold L and Radford H., *Br. J. Cancer.*, 74:722-727 (1996)

Woodget JR, Gould KL and Hunter T, *Eur. J. Biochem.*, **161**:177-184 (1986)

CLAIMS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence encoding ELF5 wherein said ELF5 comprises an Ets domain.

2. An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>2 or a derivative homologue or mimetic thereof or having at least about 45% or greater similarity to at least 10 contiguous amino acids in <400>2.

3. An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence substantially as set forth in <400>1 or a derivative or homologue thereof capable of hybridising to <400>1 under low stringency conditions.

4. An isolated nucleic acid molecule according to claim 3 which further encodes an amino acid sequence substantially as set forth in <400>2 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>2.

5. An isolated nucleic acid molecule according to claim 2 or 3 substantially as set forth in <400>1.

6. An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>4 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to at least 10 contiguous amino acids in <400>4.

7. An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence substantially as set forth in <400>3 or a derivative or homologue thereof capable of hybridising to <400>3 under low stringency conditions.

8. An isolated nucleic acid molecule according to claim 7 which further encodes an amino acid sequence substantially as set forth in <400>4 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>4.

9. An isolated nucleic acid molecule according to claim 6 or 7 substantially as set forth in <400>3.

10. An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>7 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to at least 10 contiguous amino acids in <400>7.

11. An isolated nucleic acid molecule or derivative, homologue or analogue thereof comprising a nucleotide sequence substantially as set forth in one of <400>5 or <400>6 or a derivative or homologue thereof capable of hybridising to one of <400>5 or <400>6 under low stringency conditions.

12. An isolated nucleic acid molecule according to claim 11 which further encodes an amino acid sequence corresponding to an amino acid sequence set forth in <400>7 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>7.

13. An isolated nucleic acid molecule according to claim 10 or 11 substantially as set forth in <400>5 or <400>6.

14. An isolated protein or derivative, homologue, analogue, chemical equivalent or mimetic thereof wherein said protein is ELF5 which ELF5 comprises an Ets domain.

15. An isolated protein comprising an amino acid sequence substantially as set forth in <400>2 or a derivative, homologue or mimetic thereof or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>2 or a derivative, homologue, analogue, chemical equivalent or mimetic or said protein.

16. An isolated protein according to claim 15 encoded by a nucleotide sequence substantially as set forth in <400>1 or a derivative, homologue or analogue thereof or capable of hybridising to <400>1 under low stringency conditions or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

17. An isolated protein according to claim 15 or 16 substantially as set forth in <400>2.

18. An isolated protein having an amino acid sequence substantially as set forth in <400>4 or a derivative, homologue or mimetic thereof or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>4 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

19. An isolated protein according to claim 18 encoded by a nucleotide sequence substantially as set forth in <400>3 or a derivative, homologue or mimetic thereof or capable of hybridising to <400>3 under low stringency conditions or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

20. An isolated protein according to claim 18 or 19 substantially as set forth in <400>4.

21. An isolated protein comprising an amino acid sequence substantially as set forth in <400>7 or a derivative, homologue or mimetic thereof or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>7 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

22. An isolated protein according to claim 21 encoded by a nucleotide sequence substantially as set forth in one of <400>5 or <400>6 or a derivative, homologue or mimetic thereof or capable of hybridising to one of <400>5 or <400>6 under low stringency conditions or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

23. An isolated protein according to claim 21 or 22 substantially as set forth in <400>7.

24. An isolated protein according to any one of claims 18-23 which protein is a homodimer.

25. An isolated protein according to any one of claims 18-23 which protein is a heterodimer.

26. A method of modulating expression of *ELF5* in a mammal, said method comprising contacting the *ELF5* gene with an effective amount of an agent for a time and under conditions sufficient to modulate expression of *ELF5*.

27. A method of modulating the functional activity of ELF5 in a mammal, said method comprising administering to said mammal a modulating effective amount of an agent for a time and under conditions sufficient to increase or decrease the ELF5 activity.

28. A method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of *ELF5* or sufficient to modulate the activity of ELF5.

29. A method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of a protein according to any one of claims 18-25 or a derivative, homologue, analogue, chemical equivalent or

mimetic thereof for a time and under conditions sufficient to modulate the functional activity of said cell.

30. A method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of a nucleic acid molecule according to any one of claims 1-17 or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate the functional activity of said cell.

31. A method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of *ELF5* or sufficient to modulate the activity of ELF5 wherein said *ELF5* expression product or ELF5 modulates the activity of said cell.

32. A method according to any one of claims 28-31 wherein said functional activity is proliferation.

33. A method according to claim 32 wherein said cell is a neoplastic epithelial cell said modulation is down-regulation.

34. A method according to claim 33 wherein said neoplastic epithelial cell is of breast, prostate or lung origin.

35. A method for the treatment and/or prophylaxis of a condition characterised by the aberrant, unwanted or otherwise inappropriate cellular functional activity in a mammal of treating a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of *ELF5* wherein said modulation results in modulation of cellular functional activity.

36. A method for the treatment and/or prophylaxis of a condition characterised by the aberrant, unwanted or otherwise inappropriate cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the activity of ELF5 wherein said modulation results in modulation of cellular functional activity.

37. A method for the treatment and/or prophylaxis of a condition characterised by the aberrant, unwanted or otherwise inappropriate cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of a protein according to any one of claims 18-25 or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate cellular functional activity.

38. A method for the treatment and/or prophylaxis of a condition characterised by the aberrant, unwanted or otherwise inappropriate cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of a nucleic acid molecule according to any one of claims 1-17 or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate cellular functional activity.

39. A method of treating a mammal according to any one of claims 32-35 wherein said condition is an epithelial cell malignancy.

40. A method according to claim 39 wherein said malignant epithelial cell is of breast, prostate or lung origin.

41. A method according to claim 39 or 40 wherein said functional activity is proliferation and said modulation is down-regulation.

42. Use of an agent capable of modulating the expression of *ELF5* or a derivative, homologue, analogue, chemical equivalent or mimetic thereof in the manufacture of a medicament for the modulation of cellular functional activity.

43. Use of an agent capable of modulating the activity of ELF5 or a derivative, homologue, analogue, chemical equivalent or mimetic thereof in the manufacture of a medicament for the modulation of cellular functional activity.

44. Use of ELF5 or *ELF5* or a derivative, homologue, analogue, chemical equivalent or mimetic thereof in the manufacture of a medicament for the modulation of cellular functional activity.

45. Use according to any one of claims 42-44 wherein said cell is a malignant epithelial cell.

46. Use according to claim 45 wherein said functional activity is proliferation and said modulation is down-regulation.

47. An agent for use in modulating ELF5 activity or a derivative, homologue, analogue chemical equivalent or mimetic thereof wherein modulating said ELF5 activity modulates cellular functional activity.

48. An agent for use in modulating *ELF5* expression or a derivative, homologue, analogue, chemical equivalent or mimetic thereof wherein modulating expression of said *ELF5* modulates cellular functional activity.

49. ELF5 or *ELF5* or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for use in modulating cellular functional activity.

50. A pharamceutical composition comprising *ELF5*, ELF5 or an agent capable of modulating *ELF5* expression or ELF5 activity or derivative, homologue, analogue, chemical equivalent or mimetic thereof together with one or more pharmaceutically acceptable carriers and/or diluents.

51. An isolated antibody directed to the protein according to any one of claims 18-25.

52. An isolated antibody directed to the nucleic acid molecule according to any one of claims 1-17.

53. The antibody according to claim 51 or 52 wherein said antibody is a monoclonal antibody.

54. The antibody according to claim 51 or 52 wherein said antibody is a polyclonal antibody.

55. A method of diagnosing or monitoring a mammalian disease condition, which disease condition is characterised by aberrant cellular functional activity, said method comprising screening for ELF5 or *ELF5* in a biological sample isolated from said mammal.

56. A method for detecting an agent capable of modulating the function of ELF5 or its functional equivalent or derivative thereof said method comprising contacting a cell or extract thereof containing said ELF5 or its functional equivalent or derivative with a putative agent and detecting an altered expression phenotype associated with said ELF5 or its functional equivalent or derivative.

57. A method for detecting an agent capable of modulating the function of ELF5 or its functional equivalent or derivative thereof said method comprising contacting an epithelial cell containing said ELF5 or its functional equivalent or derivative with a putative agent and detecting an altered proliferation rate.

58. A method for detecting an agent capable of binding or otherwise associating with an ELF5 binding site or functional equivalent or derivative thereof said method comprising contacting a cell containing said ELF5 binding site or functional equivalent or derivative thereof with a putative agent and detecting an altered expression phenotype associated with modulation of the function of ELF5 or its functional equivalent or derivative.

```
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tgccttctcttgccttgaaagccttctgtctggac                            91
                                   ctagccaccacttgtcttcacggtg    116

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Figure 1a

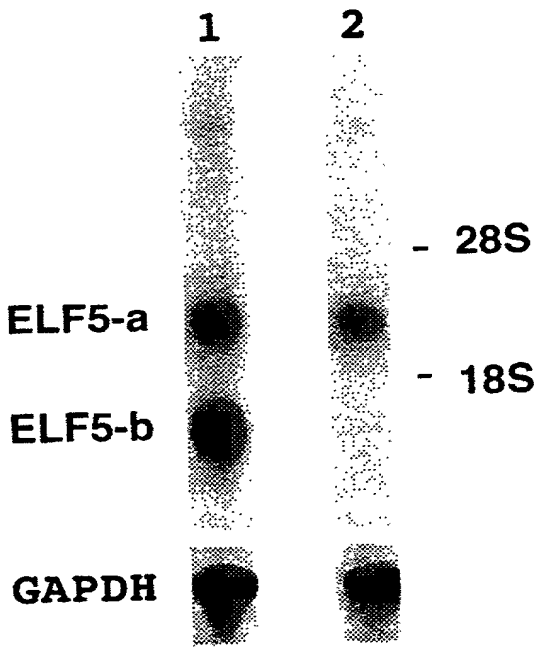

Figure 1b

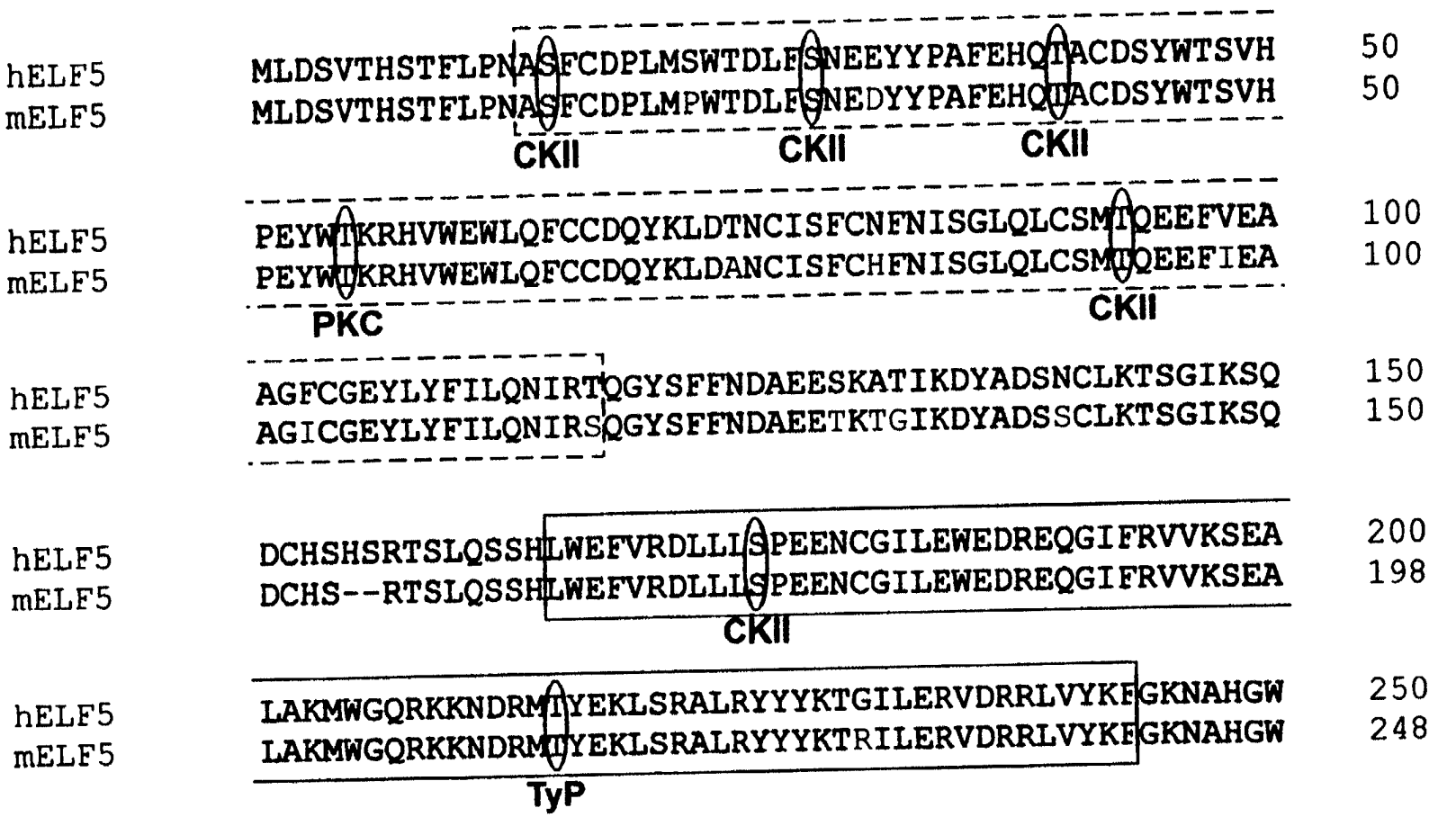

Figure 2a

PCT/AU99/00691

		IDENTITY (%)
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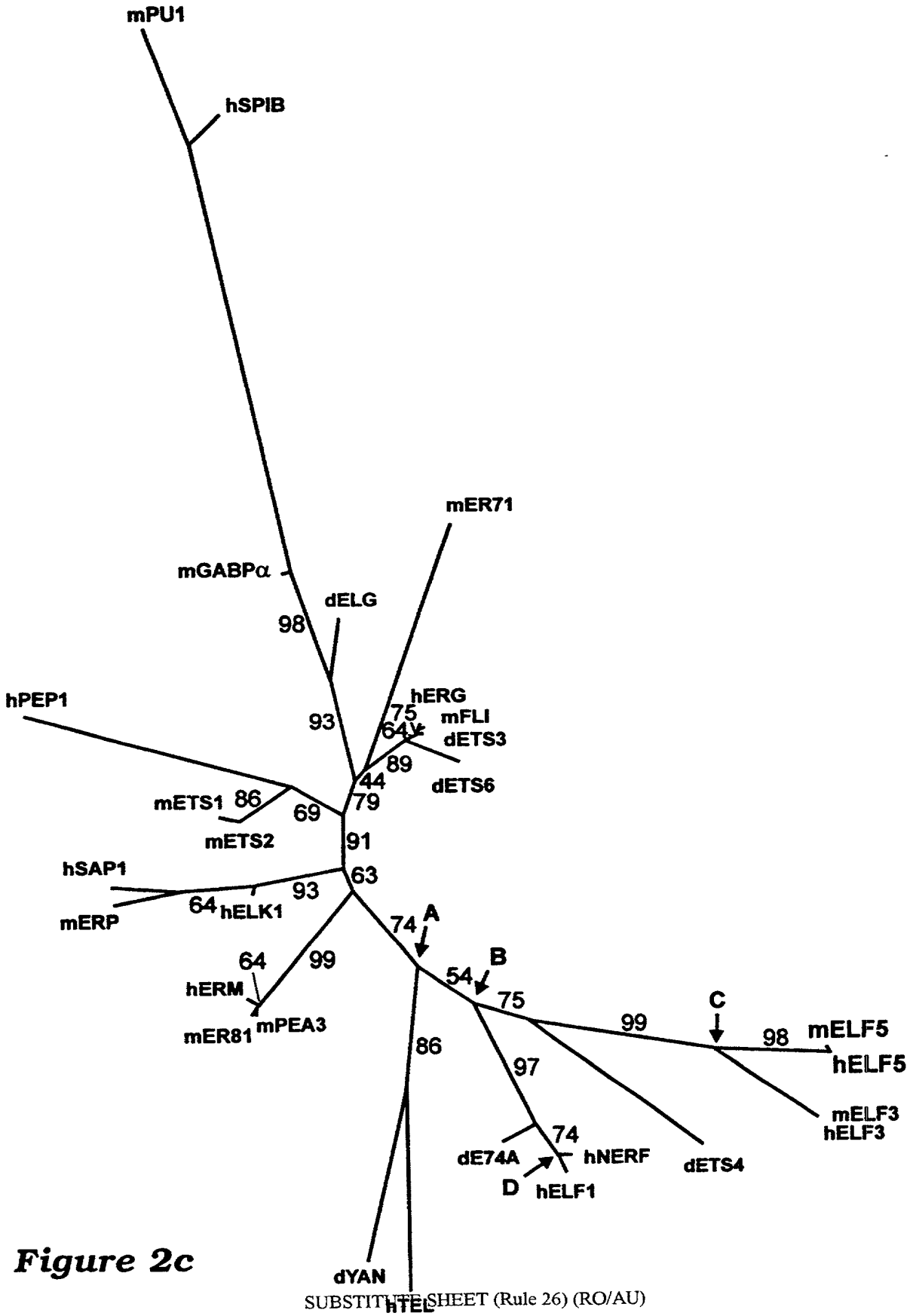
mELF5	LWEFVRDLLLSPE-ENCGILEWEDREQGIFRVV--KSEALAKMWGQRK-KNDRMTYEKLSRALRYYYKTRILERVD--RRLVYKF	98
hELF3	LWEFIRDILIHPE-LNEGLMKWENRHEGVFKFL--RSEAVAQLWGQKK-KNSNMTYEKLSRAMRYYYKREILERVD-GRRLVYKF	67
mELF3	LWEFIRDILIHPE-LNEGLMKWENRHEGVFKFL--RSEAVAQLWGQKK-KNSNMTYEKLSRAMRYYYKREILERVD-GRRLVYKF	67
hNERF	LWEFLLDLLQDKN-TCPRYIKWTQREKGIFKLV--DSKAVSKLWGKHK-NKPDMNYETMGRALRYYYQRGILAKVE-GQRLVYQF	49
dETS4	LWQFLKELLASPQ-VNGTAIRWIDRSKGIFKIE--DSVRVAKLWGRRK-NRPAMNYDKLSRSIRQYYKKGIMKKTERSQRLVYQF	48
dE74A	LWEFLLKLLQDRE-YCPRFIKWTNREKGVFKLV--DSKAVSRLWGMHK-NKPDMNYETMGRALRYYYQRGILAKVD-GQRLVYHF	48
hELF1	LWEFLLALLQDKA-TCPKYIKWTQREKGIFKLV--DSKAVSRLWGKHK-NKPDMNYETMGRALRYYYQRGILAKVE-GQRLVYQF	46
hELK1	LWQFLLQLLREQ--GNGHIISWTSRDGGEFKLV--DAEEVARLWGLRK-NKTNMNYDKLSRALRYYYDKNIIRKVS-GQKFVYKF	46
hTEL	LWDYVYQLLSDS--RYENFIRWEDKESKIFRIV--DPNGLARLWGNHK-NRTNMTYEKMSRALRHYYKLNIIRKEP-GQRLLFRF	44
hERM	LWQFLVTLLDDP--ANAHFIAWTGR-GMEFKLI--EPEEVARRWGIQK-NRPAMNYDKLSRSLRYYYEKGIMQKVA-GERYVYKF	44
mER81	LWQFLVALLDDP--SNSHFIAWTGR-GMEFKLI--EPEEVARRWGIQK-NRPAMNYDKLSRSLRYYYEKGIMQKVA-GERYVYKF	44
mPEA3	LWQFLVALLDDP--TNAHFIAWTGR-GMEFKLI--EPEEVARLWGIQK-NRPAMNYDKLSRSLRYYYEKGIMQKVA-GERYVYKF	44
mGABPα	LWQFLLEILTDK--DARDCISWVGDEG-EFKLN--QPELVAQKWGQRK-NKPTMNYEKLSRALRYYYDGDMICKVQ-GKRFVYKF	44
mERP	LWQFLLHLLLDQ--KHEHLICWTSNDG-EFKLL--KAEEVAKLWGLRK-NKTNMNYDKLSRALRYYYDKNIIKKVI-GQKFVYKF	42
dETS6	LWQFLLELLADS--SNANAISWEGQSG-EFRLI--DPDEVARRWGERK-AKPNMNYDKLSRALRYYYDKNIMTKVH-GKRYAYKF	42
mPU1	LYQFLLDLLRSG--DMKDSIWWVDKDKGTFQFSSKHKEALAHRWGIQKGNRKKMTYQKMARALRNYGKTGEVKKVK--KKLTYQF	42
hPE1	LWHFILELLQKE--EFRHVIAWQQGEYGEFVIK--DPDEVARLWGRRK-CKPQMNYDKLSRALRYYYNKRILHKTK-GKRFTYKF	42
hSAP1	LWQFLLQLLQKP--QNKHMICWTSNDG-QFKLL--QAEEVARLWGIRK-NKPNMNYDKLSRALRYYYVKNIIKKVN-GQKFVYKF	42
hSPIB	LYQFLLGLLTRG--DMRECVWWVEPGAGVFQFSSKHKELLARRWGQQKGNRKRMTYQKLARALRNYAKTGEIRKVK--RKLTYQF	42
dYAN	LWDFLQQLLNDRNQKYSDLIAWKCRDTGVFKIV--DPAGLAKLWGIQK-NHLSMNYDKMSRALRYYYRVNILRKVQ-GERHCYQF	41
hERG	LWQFLLELLSDS--SNSSCITWEGTNG-EFKMT--DPDEVARRWGERK-SKPNMNYDKLSRALRYYYDKNIMTKVH-GKRYAYKF	41
mFLI1	LWQFLLELLSDS--ANASCITWEGTNG-EFKMT--DPDEVARRWGERK-SKPNMNYDKLSRALRYYYDKNIMTKVH-GKRYAYKF	41
dELG	LWQFLLEILTDC--EHTDVIEWVGTEG-EFKLT--DPDRVARLWGEKK-NKPAMNYEKLSRALRYYYDGDMISKVS-GKRFAYKF	40
dETS3	LWQFLLELLSDS--NNASCITWEGTNG-EFKLT--DPDEVARRWGERK-SKPNMNYDKLSRALR----------	39
mETS1	LWQFLLELLTDK--SCQSFISWTGDGW-EFKLS--DPDEVARRWGKRK-NKPKMNYEKLSRGLRYYYDKNIIHKTA-GKRYVYRF	37
mETS2	LWQFLLELLSDK--SCQSFISWTGDGW-EFKLA--DPDEVARRWGKRK-NKPKMNYEKLSRGLRYYYDKNIIHKTS-GKRYVYRF	37
mER71	LWQFLLKLLQDG--ARSSCIRWTGNSR-EFQLC--DPKEVARLWGERK-RKPGMNYEKLSRGLRYYYRRDIVLKSG-GRKYTYRF	36
consensus	LWQFLL LL D I W FK VAR WG K P MNY KLSR LRYYY I K GR Y F	

Figure 2b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89
44
dETS6
mETS1
86
79
69
mETS2
91
hSAP1
63
93
64
hELK1
mERP
74
A
64
99
B
54
hERM
75
C
99
98
mELF5
mER81
mPEA3
86
97
hELF5
mELF3
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D
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dYAN
hTEL

Figure 2c

PCT/AU99/00691

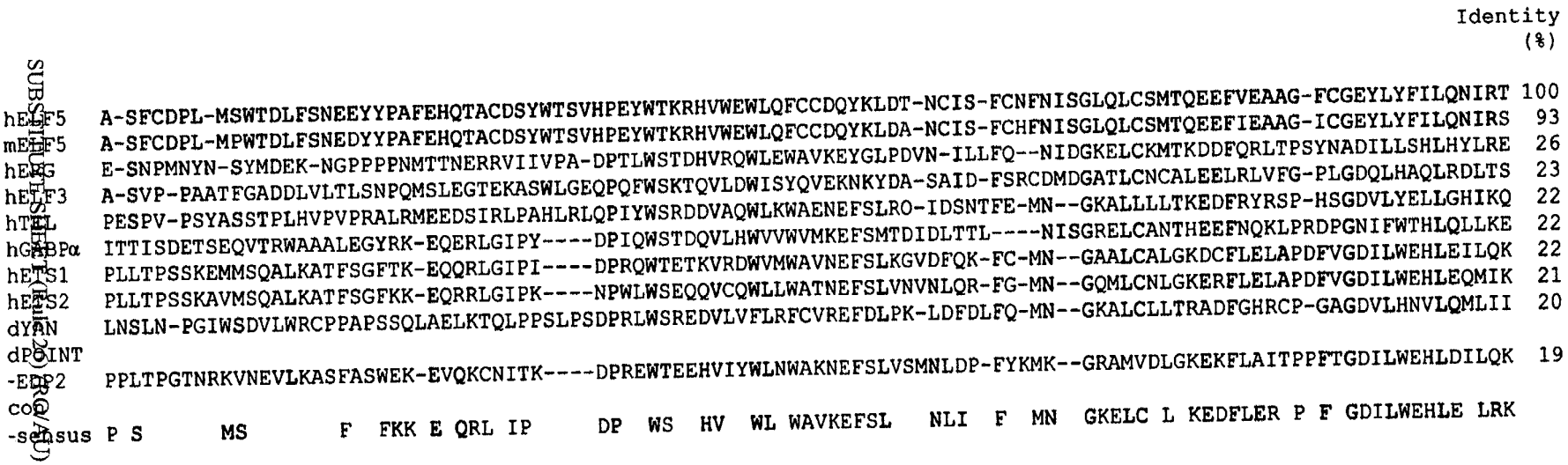
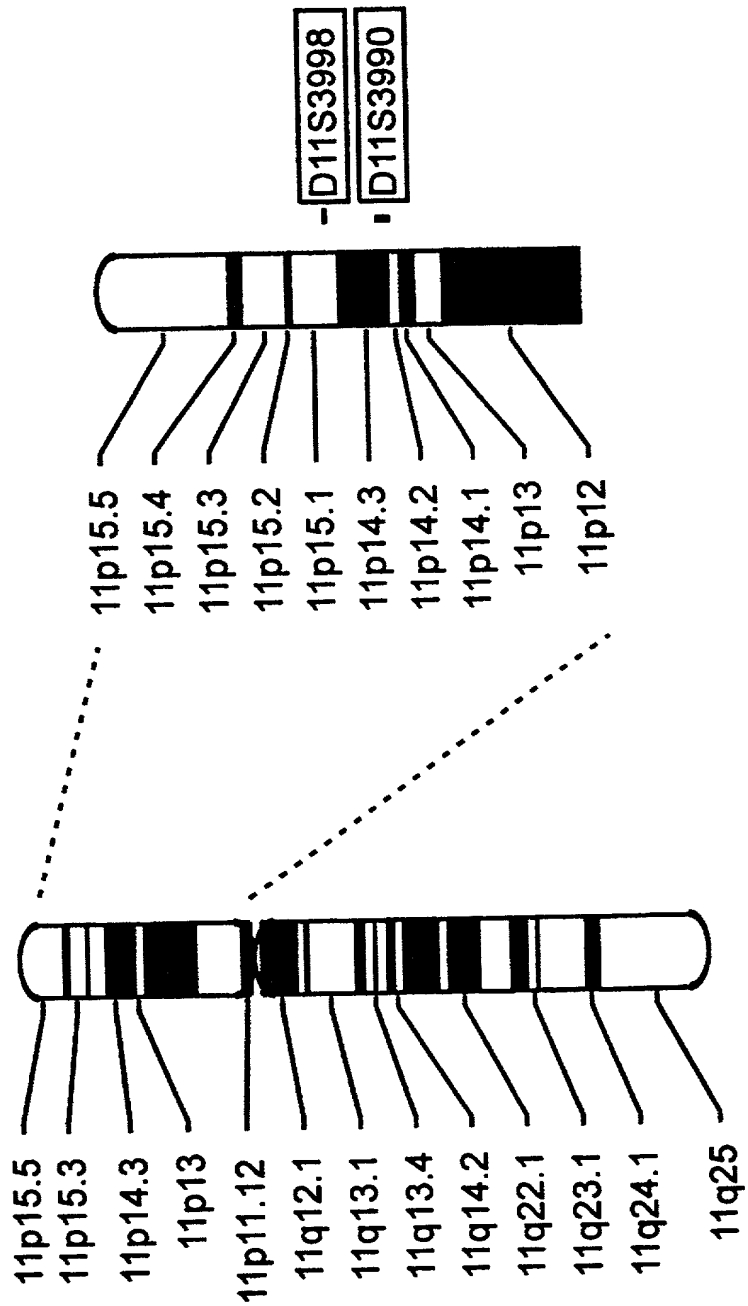


Figure 2d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11p13
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11p15.5
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11p14.3
11p13
11p11.12
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11q24.1
11q25

Figure 3

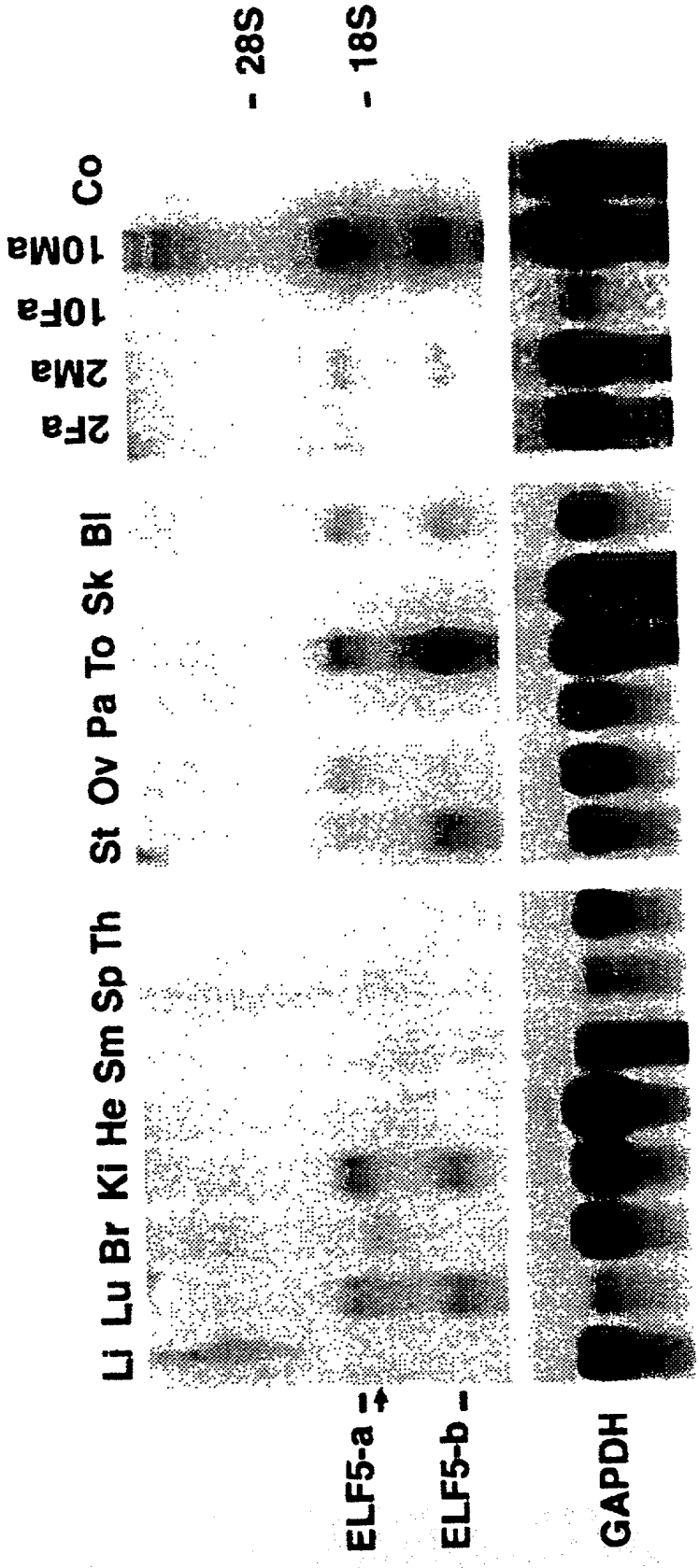

- 28S
- 18S
Co
10Ma
10Fa
2Ma
2Fa
St Ov Pa To Sk Bl
Li Lu Br Ki He Sm Sp Th
ELF5-a
ELF5-b
GAPDH

Figure 4a

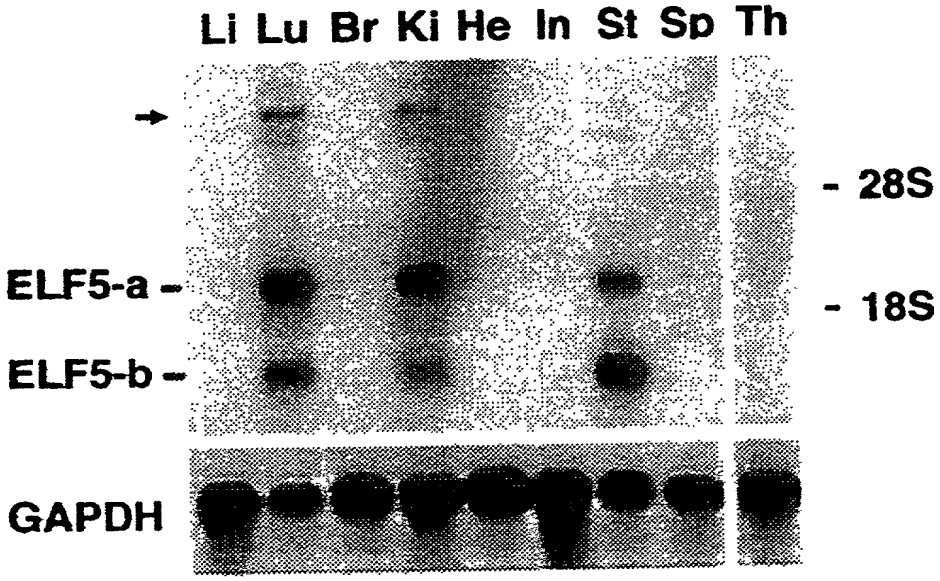
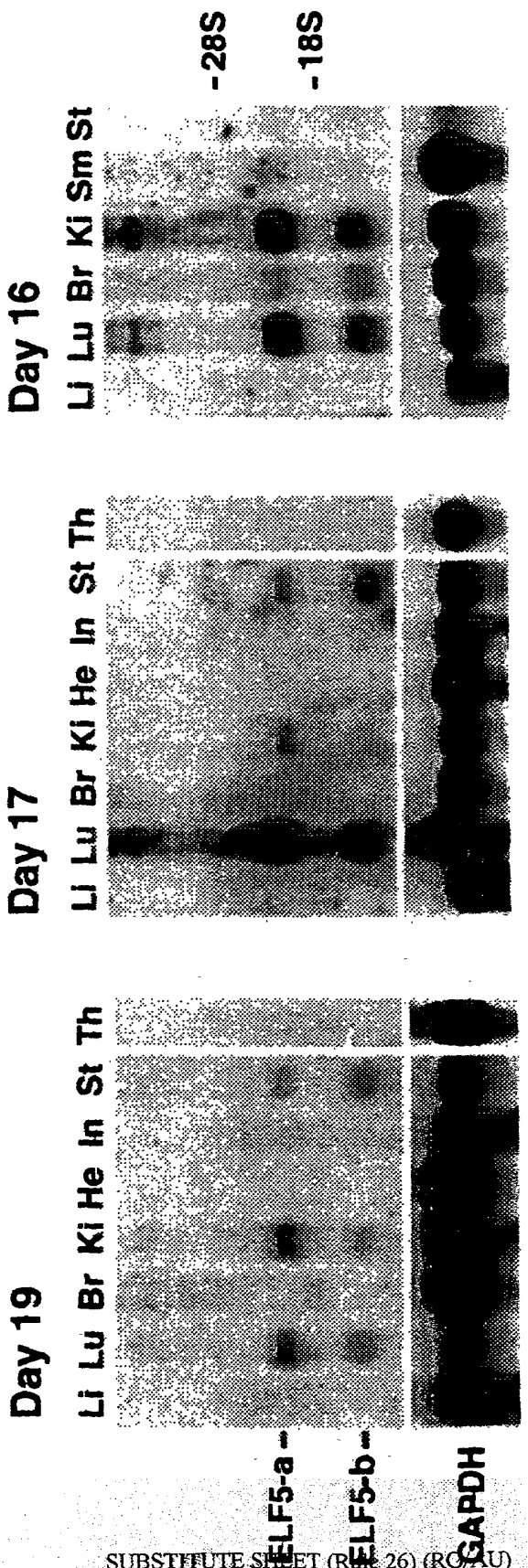


Figure 4b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-18S
Day 16
Li Lu Br Ki Sm St
Day 17
Li Lu Br Ki He In St Th
Day 19
Li Lu Br Ki He In St Th
ELF5-a
ELF5-b
GAPDH

Figure 4c

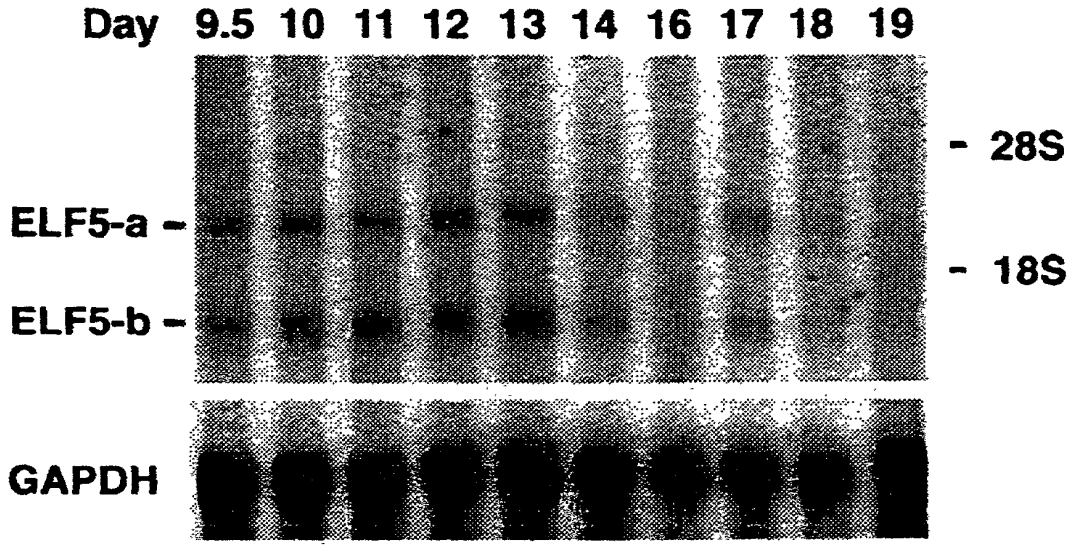
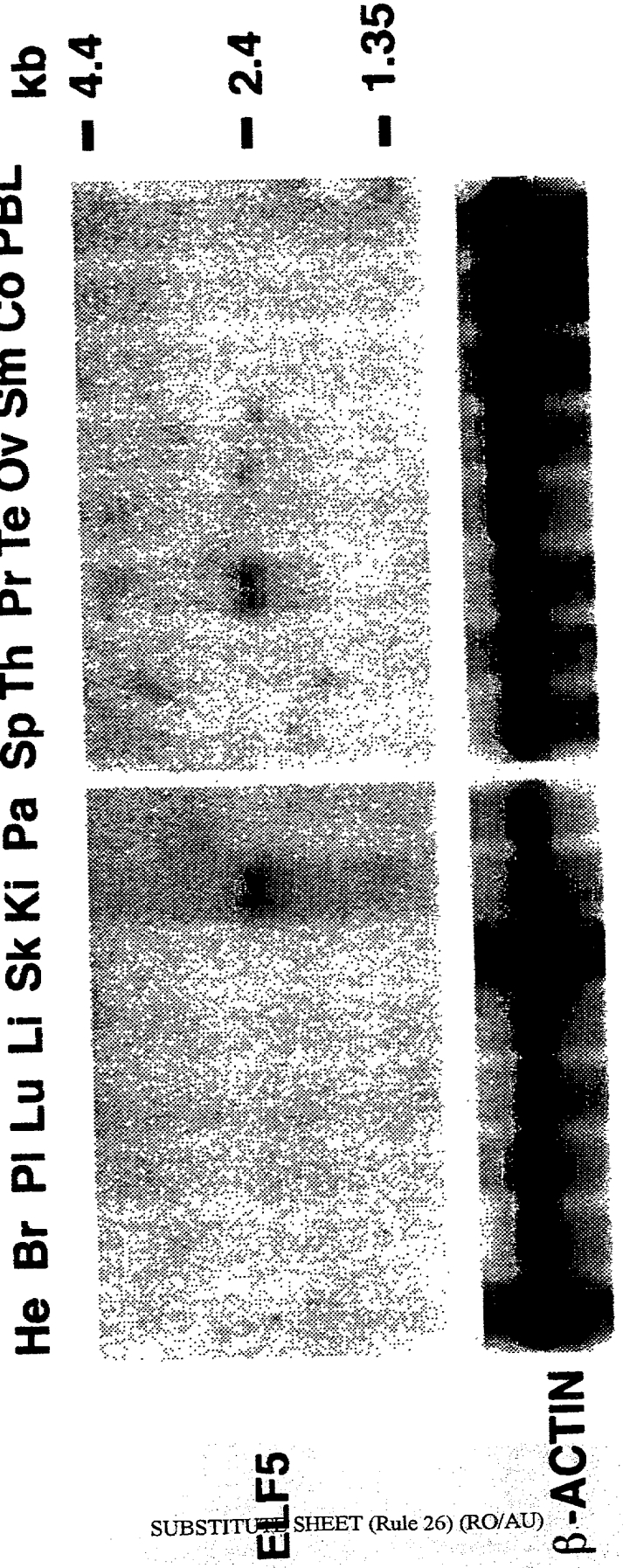


Figure 4d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2.4
1.35
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ELF5
β-ACTIN

Figure 5a

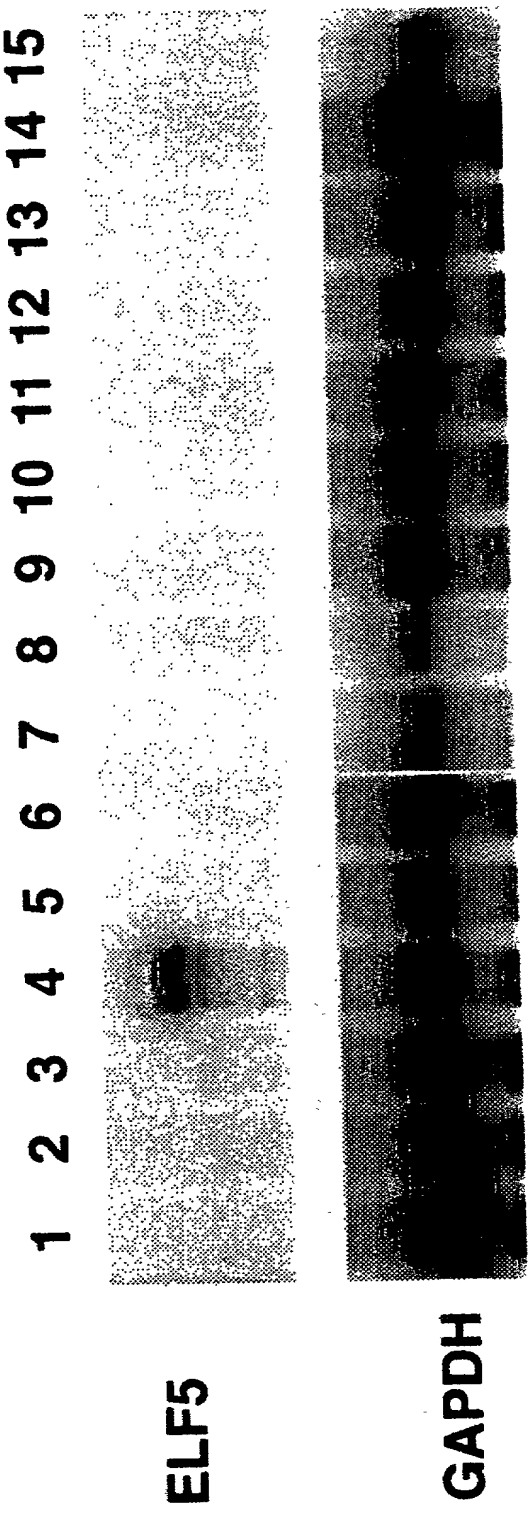
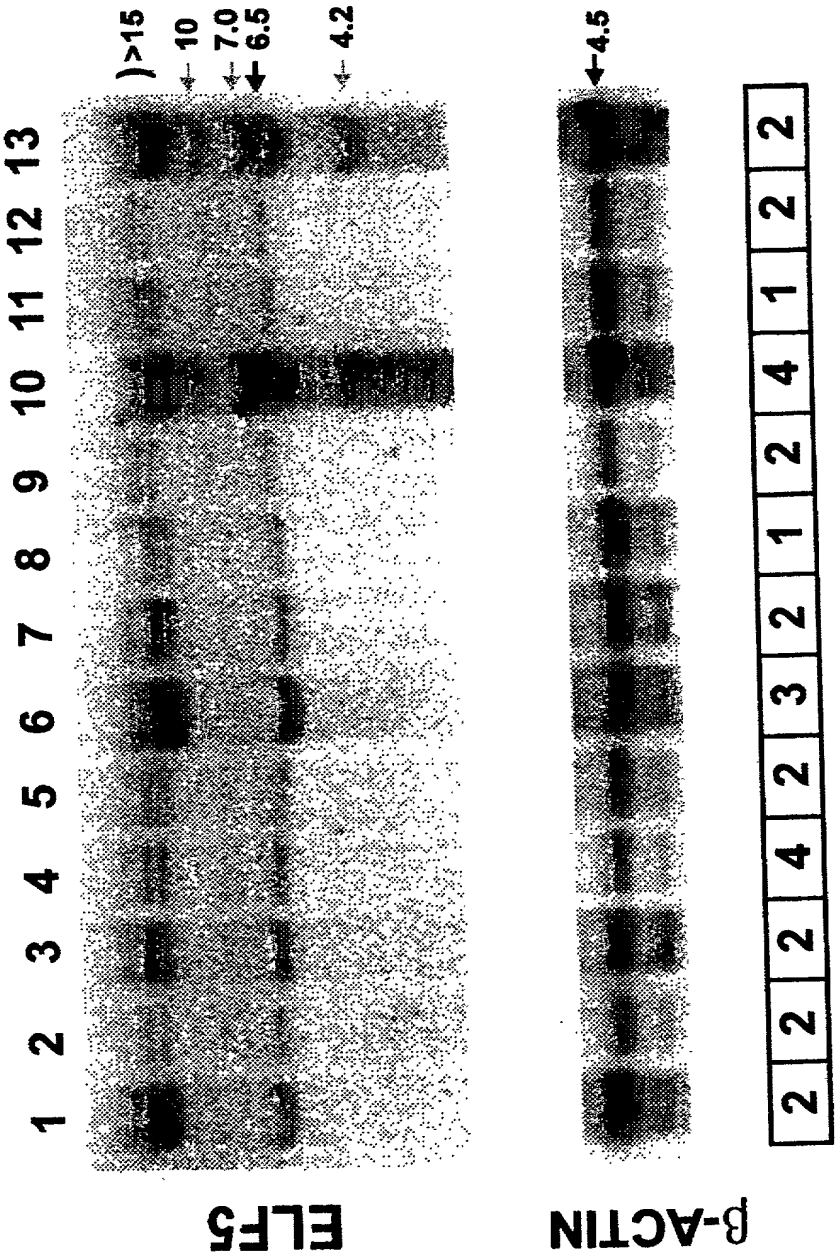


Figure 5b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6.5
4.2
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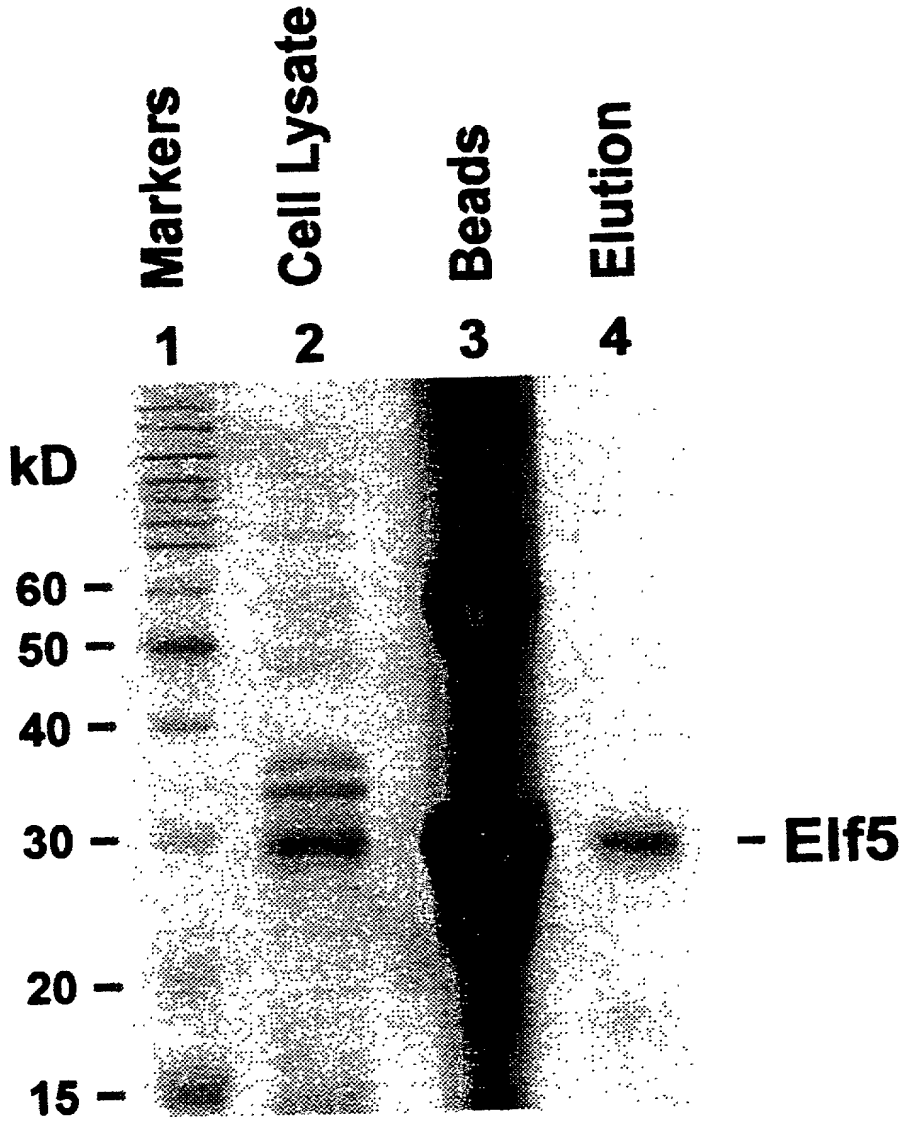
ELF5
β-ACTIN

Figure 5c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Beads
Elution
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3
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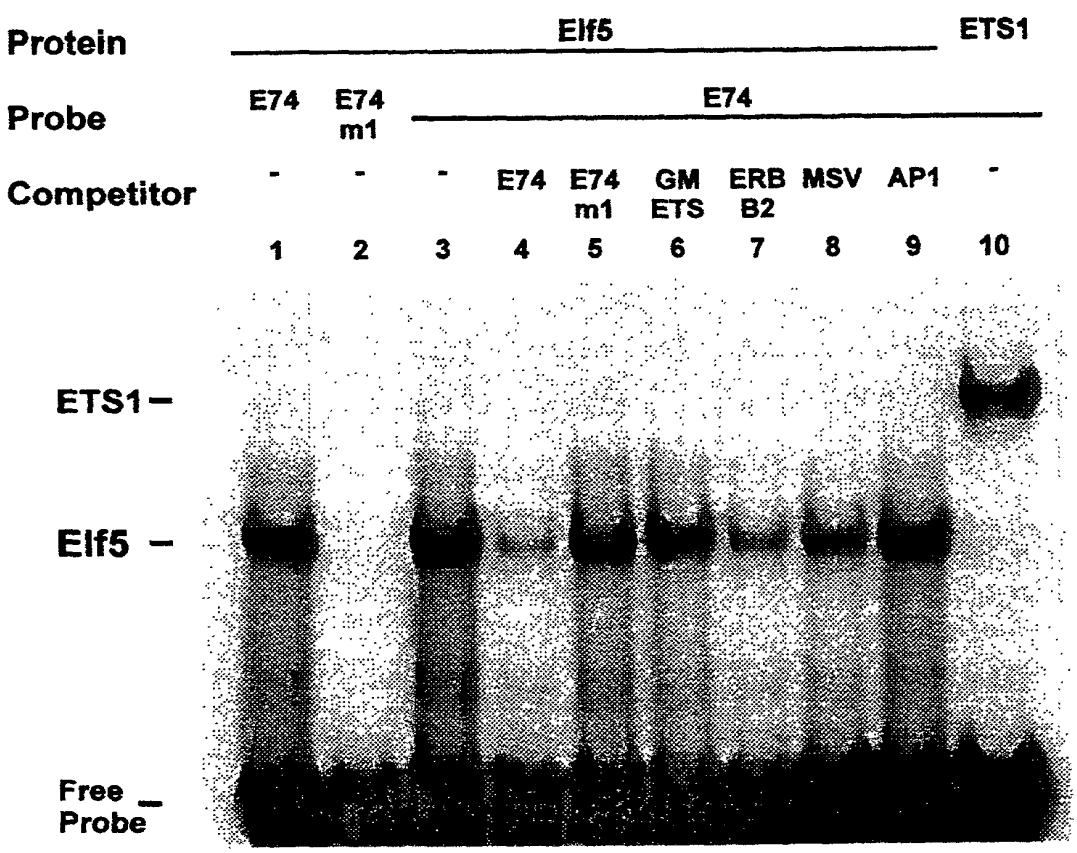
kD
60
50
40
30
20
15
Elf5

Figure 6a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ETS1
Probe
E74
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Competitor
-
-
-
E74
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GM ETS
ERB B2
MSV
AP1
-
1
2
3
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5
6
7
8
9
10
ETS1
Elf5
Free Probe

Figure 6b

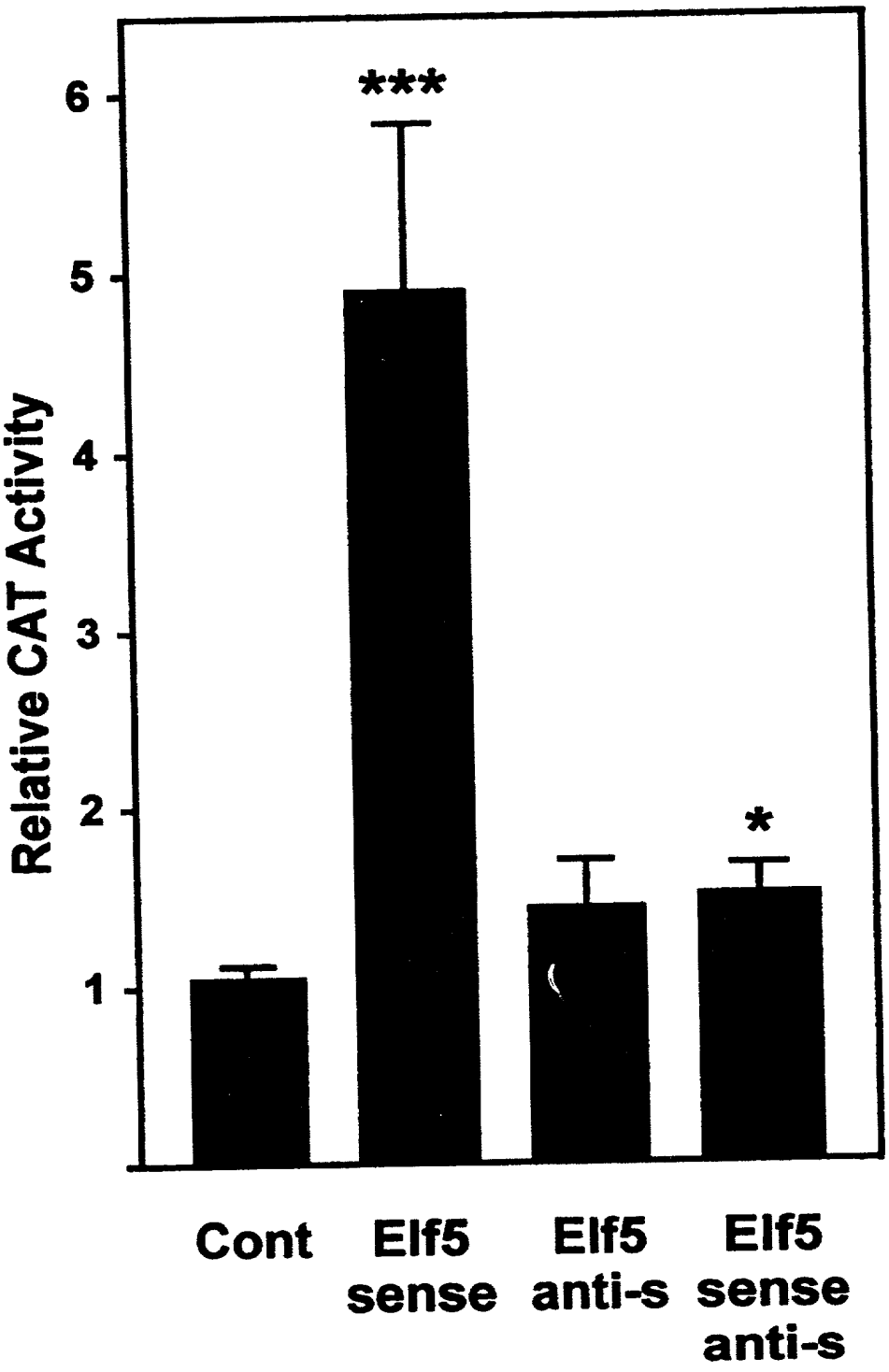
***
*
Relative CAT Activity
1
2
3
4
5
6
Cont
Elf5 sense
Elf5 anti-s
Elf5 sense anti-s

Figure 7

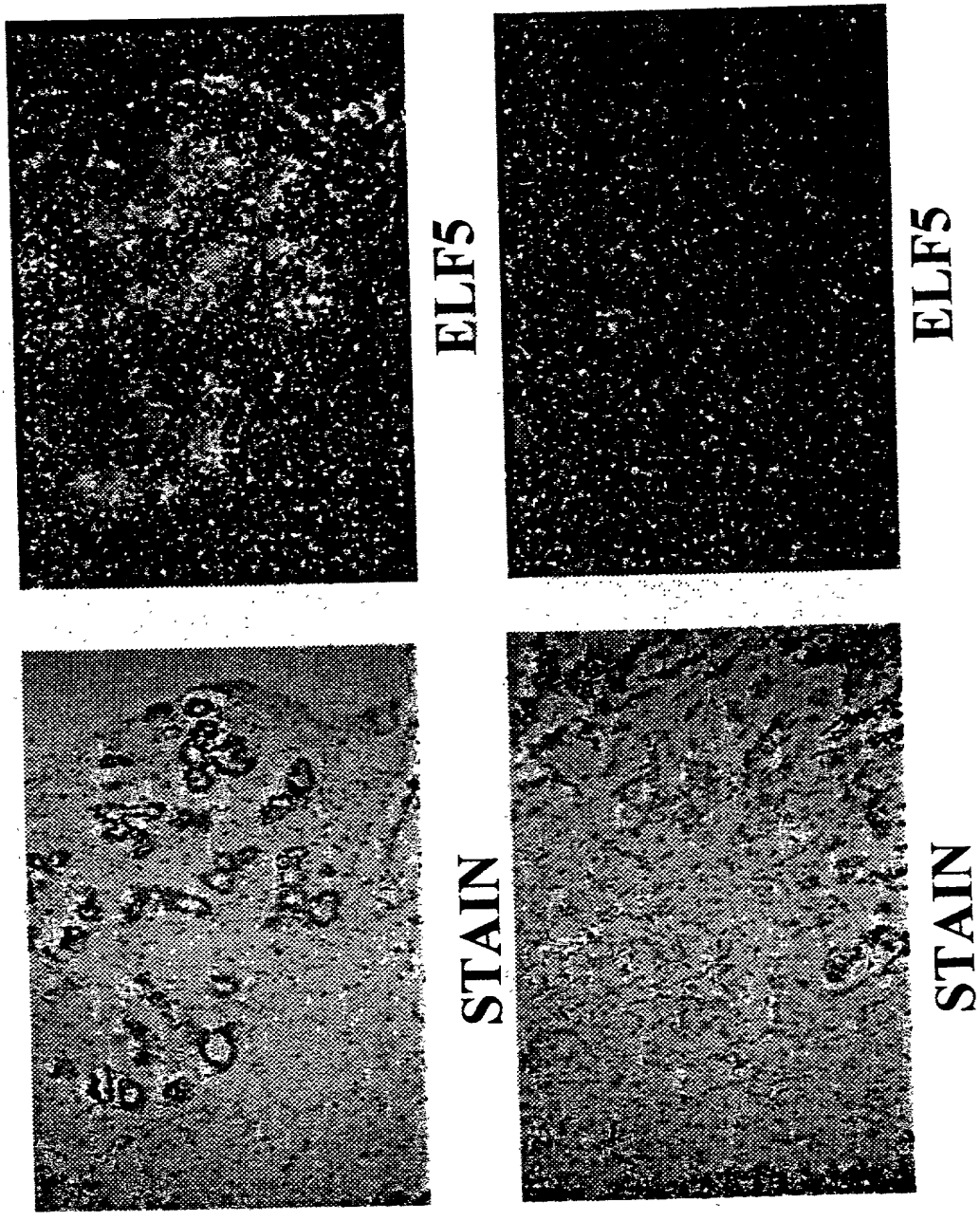

Figure 8

Attorney's Docket No. _____

DECLARATION AND POWER OF ATTORNEY - USA PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled NOVEL THERAPEUTIC MOLECULES AND USES THEREFOR

the specification of which:

(a) ☐ is attached hereto; or

(b) ☐ was filed on _____ as ☐ Application No. 0 /_____ or ☐ Express Mail No., as Application No. not yet known _____ and was amended on _____ (if applicable); or

(c) ☒ was described and claimed in PCT International Application No. PCT/AU99/00691 filed on 26 August, 1999 and as amended under PCT Article 19 on _____ (if any) and/or under PCT Article 34 on _____ (if any)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above;

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56;

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent, design or inventor's certificate or any PCT international application(s) listed below and have also identified below any foreign application(s) for patent, design or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed for the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

COUNTRY (OR INDICATE IF PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 37 U.S.C. § 119
Australia	PP5512/98	27 August, 1998	☒ YES NO ☐
Australia	PP6252/98	30 September,1998	☒ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below, and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56, which became available between the filing date of the prior application and the national or PCT international filing date of this application:

KNOBBE, MARTENS, OLSON & BEAR, LLP
620 NEWPORT CENTER DR. 16TH FLOOR NEWPORT BEACH, CA 92660
(714) 760-0404 FAX (714) 760-9502

Prior U.S.A. Application(s)

Application No.: _____ Filing Date: _____ Status: _____

POWER OF ATTORNEY: I hereby appoint the registrants of Knobbe, Martens, Olson & Bear, LLP, 620 Newport Center Drive, Sixteenth Floor, Newport Beach, California 92660, Telephone (714) 760-0404, **Customer No. 20,995**, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith (if this application is assigned, I acknowledge that the appointed individuals do not represent me, and that instead they represent the assignee).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor: Ismail KOLA

Inventor's signature _____ Day 21 Month May Year 2001

Residence (city and country): ~~Armadale, Victoria, Australia~~ Glencoe, Illinois, U.S.A

Citizenship: Australian IL

Post Office Address: ~~593 Orrong Road, Armadale, Victoria 3143, Australia~~
120 Dell Place, Glencoe, Illinois, U.S.A 60022

Full name of second inventor: Jiong ZHOU

Inventor's signature _____ Day 1 Month 5 Year 2001

Residence (city and country): Vermont South, Victoria, Australia

Citizenship: Australian AUX

Post Office Address: 5 Valerie Court, Vermont South, Victoria 3133, Australia

Full name of third inventor: _____

Inventor's signature _____ Day ___ Month _____ Year _____

Residence (city and country): _____

Citizenship: _____

Post Office Address: _____

Send Correspondence To:
KNOBBE, MARTENS, OLSON & BEAR, LLP
Customer No. 20,995

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL-ENTITY STATUS

1. I, the undersigned, do hereby declare that:

a. [ ] **I am an independent inventor** as defined in 37 CFR 1.9(c) for purposes of paying reduced fees to the Patent and Trademark Office with regard to the invention described in the patent or application identified above; OR

b. [ ] **While I am not an inventor, I declare that rights under contract or law have been conveyed to and remain with me with regard to the invention described in the patent or application identified above**. I would qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying fees to the United States Patent and Trademark Office if I had made the invention; OR

c. [ ] **I am the owner of the small business concern** identified below OR
[ ] **I am an official of the small business concern** empowered to act on behalf of the concern identified below:

NAME OF SMALL BUSINESS: _____
ADDRESS OF SMALL BUSINESS: _____

If either of the boxes in item (c) is checked, I further declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.1301 through 121.1305, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both. I further declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention described in the patent or application identified above; OR

d. [X] **I am an official empowered to act on behalf of the nonprofit organization** identified below:

NAME OF NONPROFIT ORGANIZATION: MONASH UNIVERSITY
ADDRESS OF NONPROFIT ORGANIZATION: ~~Level 5, Monash Medical Centre,~~ ~~246 Clayton Road,~~ MMD Wellington Road, Clayton, Victoria ~~3168,~~ 3800
TYPE OF NONPROFIT ORGANIZATION: Australia

[X] university or other institution of higher education; OR
[ ] tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3)); OR
[ ] nonprofit scientific or educational organization qualified under a nonprofit organization statute under a statute of a state of the United States of America
(name of state: _____)
(citation of statute: _____); OR
[ ] would qualify as tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3)) if located in the United States of America; OR
[ ] would qualify as nonprofit scientific or educational organization qualified under a nonprofit organization statute under a statute of a state of the United States of America if located in the United States of America
(name of state: _____)
(citation of statute: _____)

If Box (d) is checked, I further declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees to the United States Patent and Trademark Office regarding the invention described in the patent or application identified above.

2. The individual, concern or organization identified above has not assigned, granted, conveyed or licensed, and is under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

3. If the rights held by the above-identified individual, concern or organization are not exclusive, each individual, concern or organization having rights in the invention are identified below. Each such individual, concern or organization must file separate verified statements averring to their status as small entities.

***NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27).**

FULL NAME: _____
ADDRESS: _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

FULL NAME: _____
ADDRESS: _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

4. I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small-entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: PETER Leo DARVALL.
TITLE OF PERSON (if not an owner or individual): Deputy Vice-Chancellor
ADDRESS OF PERSON SIGNING: Wellington Road, Clayton, VIC 3800 AUSTRALIA

NAME OF PERSON SIGNING: _____
TITLE OF PERSON (if not an owner or individual): _____
ADDRESS OF PERSON SIGNING: _____

NAME OF PERSON SIGNING: _____
TITLE OF PERSON (if not an owner or individual): _____
ADDRESS OF PERSON SIGNING: _____

SIGNATURE: _____ DATE: 8 - 5 - 2001.

SIGNATURE: _____ DATE: _____

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Tyr	Pro	Ala	Phe	Glu	His	Gln	Thr	Ala	Cys	Asp	Ser	Tyr	Trp	Thr	Ser
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Val	His	Pro	Glu	Tyr	Trp	Thr	Lys	Arg	His	Val	Trp	Glu	Trp	Leu	Gln
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Phe	Cys	Cys	Asp	Gln	Tyr	Lys	Leu	Asp	Ala	Asn	Cys	Ile	Ser	Phe	Cys
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His	Phe	Asn	Ile	Ser	Gly	Leu	Gln	Leu	Cys	Ser	Met	Thr	Gln	Glu	Glu
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Gln Asn Ile Arg Ser Gln Gly Tyr Ser Phe Phe Asn Asp Ala Glu Glu
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Thr Lys Thr Gly Ile Lys Asp Tyr Ala Asp Ser Ser Cys Leu Lys Thr
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Ser Gly Ile Lys Ser Gln Asp Cys His Ser Arg Thr Ser Leu Gln Ser
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Ser His Leu Trp Glu Phe Val Arg Asp Leu Leu Leu Ser Pro Glu Glu
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Asn Cys Gly Ile Leu Glu Trp Glu Asp Arg Glu Gln Gly Ile Phe Arg
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Val Val Lys Ser Glu Ala Leu Ala Lys Met Trp Gly Gln Arg Lys Lys
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Asn Asp Arg Met Thr Tyr Glu Lys Leu Ser Arg Ala Leu Arg Tyr Tyr
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